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(57) Abstract

Invertebrate and vertebrate *patched* genes are provided, including the mouse and human *patched* genes, as well as methods for isolation of related genes, where the genes may be of different species or in the same family. Having the ability to regulate the expression of the *patched* gene, allows for the elucidation of embryonic development, cellular regulation associated with signal transduction by the *patched* gene, the identification of agonist and antagonist to signal transduction, identification of ligands for binding to *patched*, isolation of the ligands, and assaying for levels of transcription and expression of the *patched* gene.

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## PATCHED GENES AND THEIR USE

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### INTRODUCTION

#### Technical Field

The field of this invention concerns segment polarity genes and their uses.

#### 10 Background

Segment polarity genes were discovered in flies as mutations which change the pattern of structures of the body segments. Mutations in the genes cause animals to develop the changed patterns on the surfaces of body segments, the changes affecting the pattern along the head to tail axis. For example, mutations in the gene

15 *patched* cause each body segment to develop without the normal structures in the center of each segment. In their stead is a mirror image of the pattern normally found in the anterior segment. Thus cells in the center of the segment make the wrong structures, and point them in the wrong direction with reference to the over all head-to-tail polarity of the animal. About sixteen genes in the class are known.

20 The encoded proteins include kinases, transcription factors, a cell junction protein, two secreted proteins called wingless (WG) and hedgehog (HH), a single transmembrane protein called *patched* (PTC), and some novel proteins not related to any known protein. All of these proteins are believed to work together in signaling pathways that inform cells about their neighbors in order to set cell fates and

25 polarities.

Many of the segment polarity proteins of *Drosophila* and other invertebrates are closely related to vertebrate proteins, implying that the molecular mechanisms involved are ancient. Among the vertebrate proteins related to the fly genes are En-1 and -2, which act in vertebrate brain development and WNT-1, which is also  
5 involved in brain development, but was first found as the oncogene implicated in many cases of mouse breast cancer. In flies, the *patched* gene is transcribed into RNA in a complex and dynamic pattern in embryos, including fine transverse stripes in each body segment primordium. The encoded protein is predicted to contain many transmembrane domains. It has no significant similarity to any other known  
10 protein. Other proteins having large numbers of transmembrane domains include a variety of membrane receptors, channels through membranes and transporters through membranes.

The hedgehog (HH) protein of flies has been shown to have at least three vertebrate relatives: *Sonic hedgehog* (*Shh*); *Indian hedgehog*, and *Desert hedgehog*.  
15 The *Shh* is expressed in a group of cells at the posterior of each developing limb bud. This is exactly the same group of cells found to have an important role in signaling polarity to the developing limb. The signal appears to be graded, with cells close to the posterior source of the signal forming posterior digits and other limb structures and cells farther from the signal source forming more anterior  
20 structures. It has been known for many years that transplantation of the signaling cells, a region of the limb bud known as the "zone of polarizing activity (ZPA)" has dramatic effects on limb patterning. Implanting a second ZPA anterior to the limb bud causes a limb to develop with posterior features replacing the anterior ones (in essence little fingers instead of thumbs). *Shh* has been found to be the long sought  
25 ZPA signal. Cultured cells making *Shh* protein (SHH), when implanted into the anterior limb bud region, have the same effect as an implanted ZPA. This establishes that *Shh* is clearly a critical trigger of posterior limb development.

The factor in the ZPA has been thought for some time to be related to another important developmental signal that polarizes the developing spinal cord.  
30 The notochord, a rod of mesoderm that runs along the dorsal side of early vertebrate embryos, is a signal source that polarizes the neural tube along the dorsal-ventral axis. The signal causes the part of the neural tube nearest to the notochord to form

floor plate, a morphologically distinct part of the neural tube. The floor plate, in turn, sends out signals to the more dorsal parts of the neural tube to further determine cell fates. The ZPA was reported to have the same signaling effect as the notochord when transplanted to be adjacent to the neural tube, suggesting the ZPA makes the same signal as the notochord. In keeping with this view, *Shh* was found to be produced by notochord cells and floor plate cells. Tests of extra expression of *Shh* in mice led to the finding of extra expression of floor plate genes in cells which would not normally turn them on. Therefore *Shh* appears to be a component of the signal from notochord to floor plate and from floor plate to more dorsal parts of the neural tube. Besides limb and neural tubes, vertebrate hedgehog genes are also expressed in many other tissues including, but not limited to the peripheral nervous system, brain, lung, liver, kidney, tooth primordia, genitalia, and hindgut and foregut endoderm.

PTC has been proposed as a receptor for HH protein based on genetic experiments in flies. A model for the relationship is that PTC acts through a largely unknown pathway to inactivate both its own transcription and the transcription of the *wingless* segment polarity gene. This model proposes that HH protein, secreted from adjacent cells, binds to the PTC receptor, inactivates it, and thereby prevents PTC from turning off its own transcription or that of *wingless*. A number of experiments have shown coordinate events between PTC and HH.

#### Relevant Literature

Descriptions of *patched*, by itself or its role with *hedgehog* may be found in Hooper and Scott, Cell 59, 751-765 (1989); Nakano et al., Nature, 341, 508-513 (1989) (both of which also describes the sequence for *Drosophila patched*) Simcox et al., Development 107, 715-722 (1989); Hidalgo and Ingham, Development, 110, 291-301 (1990); Phillips et al., Development, 110, 105-114 (1990); Sampedro and Guerrero, Nature 353, 187-190 (1991); Ingham et al., Nature 353, 184-187 (1991); and Taylor et al., Mechanisms of Development 42, 89-96 (1993). Discussions of the role of *hedgehog* include Riddle et al., Cell 75, 1401-1416 (1993); Echelard et al., Cell 75, 1417-1430 (1993); Krauss et al., Cell 75, 1431-1444 (1993); Tabata and Kornberg, Cell 76, 89-102 (1994); Heemskerk & DiNardo, Cell 76, 449-460 (1994); Relink et al., Cell 76, 761-775 (1994); and a short review article by

Ingham, Current Biology 4, 347-350 (1994). The sequence for the *Drosophila* 5' non-coding region was reported to the GenBank, accession number M28418, referred to in Hooper and Scott (1989), *supra*. See also, Forbes, et al., Development 1993 Supplement 115-124.

5

### SUMMARY OF THE INVENTION

Methods for isolating *patched* genes, particularly mammalian *patched* genes, including the mouse and human *patched* genes, as well as invertebrate *patched* genes and sequences, are provided. The methods include identification of *patched* genes from other species, as well as members of the same family of proteins. The subject genes provide methods for producing the patched protein, where the genes and proteins may be used as probes for research, diagnosis, binding of *hedgehog* protein for its isolation and purification, gene therapy, as well as other utilities.

15

### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph having a restriction map of about 10kbp of the 5' region upstream from the initiation codon of *Drosophila patched* gene and bar graphs of constructs of truncated portions of the 5' region joined to  $\beta$ -galactosidase, where the constructs are introduced into fly cell lines for the production of embryos. The expression of  $\beta$ -gal in the embryos is indicated in the right-hand table during early and late development of the embryo. The greater the number of '+'s, the more intense the staining.

25

### DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Methods are provided for identifying members of the *patched* (*ptc*) gene family from invertebrate and vertebrate, e.g. mammalian, species, as well as the entire cDNA sequence of the mouse and human *patched* gene. Also, sequences for invertebrate *patched* genes are provided. The *patched* gene encodes a transmembrane protein having a large number of transmembrane sequences.

30

In identifying the mouse and human *patched* genes, primers were employed to move through the evolutionary tree from the known *Drosophila ptc* sequence. Two primers are employed from the *Drosophila* sequence with appropriate

restriction enzyme linkers to amplify portions of genomic DNA of a related invertebrate, such as mosquito. The sequences are selected from regions which are not likely to diverge over evolutionary time and are of low degeneracy.

Conveniently, the regions are the N-terminal proximal sequence, generally within the first 1.5kb, usually within the first 1kb, of the coding portion of the cDNA, conveniently in the first hydrophilic loop of the protein. Employing the polymerase chain reaction (PCR) with the primers, a band can be obtained from mosquito genomic DNA. The band may then be amplified and used in turn as a probe. One may use this probe to probe a cDNA library from an organism in a different branch of the evolutionary tree, such as a butterfly. By screening the library and identifying sequences which hybridize to the probe, a portion of the butterfly *patched* gene may be obtained. One or more of the resulting clones may then be used to rescreen the library to obtain an extended sequence, up to and including the entire coding region, as well as the non-coding 5'- and 3'-sequences. As appropriate, one may sequence all or a portion of the resulting cDNA coding sequence.

One may then screen a genomic or cDNA library of a species higher in the evolutionary scale with appropriate probes from one or both of the prior sequences. Of particular interest is screening a genomic library, of a distantly related invertebrate, *e.g.* beetle, where one may use a combination of the sequences obtained from the previous two species, in this case, the *Drosophila* and the butterfly. By appropriate techniques, one may identify specific clones which bind to the probes, which may then be screened for cross hybridization with each of the probes individually. The resulting fragments may then be amplified, *e.g.* by subcloning.

By having all or parts of the 4 different *patched* genes, in the presently illustrated example, *Drosophila* (fly), mosquito, butterfly and beetle, one can now compare the *patched* genes for conserved sequences. Cells from an appropriate mammalian limb bud or other cells expressing *patched*, such as notochord, neural tube, gut, lung buds, or other tissue, particularly fetal tissue, may be employed for screening. Alternatively, adult tissue which produces *patched* may be employed for screening. Based on the consensus sequence available from the 4 other species, one

can develop probes where at each site at least 2 of the sequences have the same nucleotide and where the site varies that each species has a unique nucleotide, inosine may be used, which binds to all 4 nucleotides.

5        Either PCR may be employed using primers or, if desired, a genomic library from an appropriate source may be probed. With PCR, one may use a cDNA library or use reverse transcriptase-PCR (RT-PCR), where mRNA is available from the tissue. Usually, where fetal tissue is employed, one will employ tissue from the first or second trimester, preferably the latter half of the first trimester or the second trimester, depending upon the particular host. The age and source of tissue will  
10      depend to a significant degree on the ability to surgically isolate the tissue based on its size, the level of expression of *patched* in the cells of the tissue, the accessibility of the tissue, the number of cells expressing *patched* and the like. The amount of tissue available should be large enough so as to provide for a sufficient amount of mRNA to be usefully transcribed and amplified. With mouse tissue, limb bud of  
15      from about 10 to 15 dpc (days post conception) may be employed.

      In the primers, the complementary binding sequence will usually be at least 14 nucleotides, preferably at least about 17 nucleotides and usually not more than about 30 nucleotides. The primers may also include a restriction enzyme sequence for isolation and cloning. With RT-PCR, the mRNA may be enriched in accordance  
20      with known ways, reverse transcribed, followed by amplification with the appropriate primers. (Procedures employed for molecular cloning may be found in *Molecular Cloning: A Laboratory Manual*, Sambrook et al., eds., Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1988). Particularly, the primers may conveniently come from the N-terminal proximal sequence or other conserved  
25      region, such as those sequences where at least five amino acids are conserved out of eight amino acids in three of the four sequences. This is illustrated by the sequences (SEQ ID NO:11) IITPLDCFWEQ, (SEQ ID NO:12) LIVGG, and (SEQ ID NO:13) PFFWEQY. Resulting PCR products of expected size are subcloned and may be sequenced if desired.

30      The cloned PCR fragment may then be used as a probe to screen a cDNA library of mammalian tissue cells expressing *patched*, where hybridizing clones may be isolated under appropriate conditions of stringency. Again, the cDNA library



should come from tissue which expresses *patched*, which tissue will come within the limitations previously described. Clones which hybridize may be subcloned and rescreened. The hybridizing subclones may then be isolated and sequenced or may be further analyzed by employing RNA blots and *in situ* hybridizations in whole and sectioned embryos. Conveniently, a fragment of from about 0.5 to 1 kbp of the N-terminal coding region may be employed for the Northern blot.

The mammalian gene may be sequenced and as described above, conserved regions identified and used as primers for investigating other species. The N-terminal proximal region, the C-terminal region or an intermediate region may be employed for the sequences, where the sequences will be selected having minimum degeneracy and the desired level of conservation over the probe sequence.

The DNA sequence encoding PTC may be cDNA or genomic DNA or fragment thereof, particularly complete exons from the genomic DNA, may be isolated as the sequence substantially free of wild-type sequence from the chromosome, may be a 50 kbp fragment or smaller fragment, may be joined to heterologous or foreign DNA, which may be a single nucleotide, oligonucleotide of up to 50 bp, which may be a restriction site or other identifying DNA for use as a primer, probe or the like, or a nucleic acid of greater than 50 bp, where the nucleic acid may be a portion of a cloning or expression vector, comprise the regulatory regions of an expression cassette, or the like. The DNA may be isolated, purified being substantially free of proteins and other nucleic acids, be in solution, or the like.

The subject gene may be employed for producing all or portions of the *patched* protein. The subject gene or fragment thereof, generally a fragment of at least 12 bp, usually at least 18 bp, may be introduced into an appropriate vector for extrachromosomal maintenance or for integration into the host. Fragments will usually be immediately joined at the 5' and/or 3' terminus to a nucleotide or sequence not found in the natural or wild-type gene, or joined to a label other than a nucleic acid sequence. For expression, an expression cassette may be employed, providing for a transcriptional and translational initiation region, which may be inducible or constitutive, the coding region under the transcriptional control of the transcriptional initiation region, and a transcriptional and translational termination

region. Various transcriptional initiation regions may be employed which are functional in the expression host. The peptide may be expressed in prokaryotes or eukaryotes in accordance with conventional ways, depending upon the purpose for expression. For large production of the protein, a unicellular organism or cells of a higher organism, *e.g.* eukaryotes such as vertebrates, particularly mammals, may be used as the expression host, such as *E. coli*, *B. subtilis*, *S. cerevisiae*, and the like. In many situations, it may be desirable to express the *patched* gene in a mammalian host, whereby the *patched* gene will be transported to the cellular membrane for various studies. The protein has two parts which provide for a total of six transmembrane regions, with a total of six extracellular loops, three for each part. The character of the protein has similarity to a transporter protein. The protein has two conserved glycosylation signal triads.

The subject nucleic acid sequences may be modified for a number of purposes, particularly where they will be used intracellularly, for example, by being joined to a nucleic acid cleaving agent, *e.g.* a chelated metal ion, such as iron or chromium for cleavage of the gene; as an antisense sequence; or the like. Modifications may include replacing oxygen of the phosphate esters with sulfur or nitrogen, replacing the phosphate with phosphoramidate, etc.

With the availability of the protein in large amounts by employing an expression host, the protein may be isolated and purified in accordance with conventional ways. A lysate may be prepared of the expression host and the lysate purified using HPLC, exclusion chromatography, gel electrophoresis, affinity chromatography, or other purification technique. The purified protein will generally be at least about 80% pure, preferably at least about 90% pure, and may be up to 100% pure. By pure is intended free of other proteins, as well as cellular debris.

The polypeptide may be used for the production of antibodies, where short fragments provide for antibodies specific for the particular polypeptide, whereas larger fragments or the entire gene allow for the production of antibodies over the surface of the polypeptide or protein, where the protein may be in its natural conformation.

Antibodies may be prepared in accordance with conventional ways, where the expressed polypeptide or protein may be used as an immunogen, by itself or

conjugated to known immunogenic carriers, *e.g.* KLH, pre-S HBsAg, other viral or eukaryotic proteins, or the like. Various adjuvants may be employed, with a series of injections, as appropriate. For monoclonal antibodies, after one or more booster injections, the spleen may be isolated, the splenocytes immortalized, and then  
5 screened for high affinity antibody binding. The immortalized cells, *e.g.* hybridomas, producing the desired antibodies may then be expanded. For further description, see *Monoclonal Antibodies: A Laboratory Manual*, Harlow and Lane eds., Cold Spring Harbor Laboratories, Cold Spring Harbor, New York, 1988. If desired, the mRNA encoding the heavy and light chains may be isolated and  
10 mutigenized by cloning in *E. coli*, and the heavy and light chains may be mixed to further enhance the affinity of the antibody. The antibodies may find use in diagnostic assays for detection of the presence of the PTC protein on the surface of cells or to inhibit the transduction of signal by the PTC protein ligand by competing for the binding site.

15 The mouse *patched* gene (SEQ ID NO:09) encodes a protein (SEQ ID NO:10) which has about 38% identical amino acids to fly PTC (SEQ ID NO:6) over about 1,200 amino acids. This amount of conservation is dispersed through much of the protein excepting the C-terminal region. The mouse protein also has a 50 amino acid insert relative to the fly protein. The human *patched* gene (SEQ ID  
20 NO:18) contains an open reading frame of about 1450 amino acids (SEQ ID NO:19) that is about 96% identical (98 % similar) to mouse *ptc* (SEQ ID NO:09). The human *patched* gene (SEQ ID NO:18), including coding and non-coding sequences, is about 89% identical to the mouse *patched* gene (SEQ ID NO:09).

The butterfly PTC homolog (SEQ ID NO:4) is 1,300 amino acids long and  
25 overall has a 50% amino acid identity (72% similarity) to fly PTC (SEQ ID NO:6). With the exception of a divergent C-terminus, this homology is evenly spread across the coding sequence. A 267bp exon from the beetle *patched* gene encodes an 89 amino acid protein fragment which was found to be 44% and 51% identical to the corresponding regions of fly and butterfly PTC respectively.

30 The mouse *ptc* message is about 8 kb long and the message is present in low levels as early as 7 dpc, the abundance increasing by 11 and 15 dpc. Northern blot indicates a clear decrease in the amount of message at 17 dpc. In the adult, PTC

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RNA is present in high amounts in the brain and lung, as well as in moderate amounts in the kidney and liver. Weak signals are detected in heart, spleen, skeletal muscle and testes.

- In mouse embryos, *ptc* mRNA is present at 7 dpc, using *in situ* hybridization. *ptc* is present at high levels along the neural axis of 8.5 dpc embryos. By 11.5 dpc, *ptc* can be detected in developing lung buds and gut, consistent with its Northern profile. In addition, the gene is present at high levels in the ventricular zone of the central nervous system as well as in the zona limitans of the prosencephalon. *ptc* is also strongly transcribed in the perichondrium condensing cartilage of 11.5 and 13.5 dpc limb buds, as well as in the ventral portion of the somites, a region which is prospective sclerotome and eventually forms bone in the vertebral column. PTC is present in a wide range of tissues from endodermal, mesodermal, as well as ectodermal origin, evidencing the fundamental role in many aspects of embryonic development, including the condensation of cartilage, the patterning of limbs, the differentiation of lung tissue, and the generation of neurons.

- The *patched* nucleic acid may be used for isolating the gene from various mammalian sources of interest, particularly primate, more particularly human, or from domestic animals, both pet and farm, e.g. lagomorpha, rodentiae, porcine, bovine, feline, canine, ovine, equine, etc. By using probes, particularly labeled probes of DNA sequences, of the *patched* gene, one may be able to isolate mRNA or genomic DNA, which may be then used for identifying mutations, particularly associated with genetic diseases, such as spina bifida, limb defects, lung defects, problems with tooth development, liver and kidney development, peripheral nervous system development, and other sites where a *patched* gene is involved in regulation.
- The subject probes can also be used for identifying the level of expression in cells associated with the testis to determine the relationship with the level of expression and sperm production.

- The gene or fragments thereof may be used as probes for identifying the 5' non-coding region comprising the transcriptional initiation region, particularly the enhancer regulating the transcription of *patched*. By probing a genomic library, particularly with a probe comprising the 5' coding region, one can obtain fragments comprising the 5' non-coding region. If necessary, one may walk the fragment to

obtain further 5' sequence to ensure that one has at least a functional portion of the enhancer. It is found that the enhancer is proximal to the 5' coding region, a portion being in the transcribed sequence and downstream from the promoter sequences. The transcriptional initiation region may be used for many purposes,  
5 studying embryonic development, providing for regulated expression of *patched* protein or other protein of interest during embryonic development or thereafter, and in gene therapy.

The gene may also be used for gene therapy, by transfection of the normal gene into embryonic stem cells or into mature cells. A wide variety of viral vectors  
10 can be employed for transfection and stable integration of the gene into the genome of the cells. Alternatively, micro-injection may be employed, fusion, or the like for introduction of genes into a suitable host cell. See, for example, Dhawan *et al.*, Science 254, 1509-1512 (1991) and Smith *et al.*, Molecular and Cellular Biology (1990) 3268-3271.

15 By providing for the production of large amounts of PTC protein, one can use the protein for identifying ligands which bind to the PTC protein. Particularly, one may produce the protein in cells and employ the polysomes in columns for isolating ligands for the PTC protein. One may incorporate the PTC protein into liposomes by combining the protein with appropriate lipid surfactants, *e.g.*  
20 phospholipids, cholesterol, etc., and sonicate the mixture of the PTC protein and the surfactants in an aqueous medium. With one or more established ligands, *e.g.* *hedgehog*, one may use the PTC protein to screen for antagonists which inhibit the binding of the ligand. In this way, drugs may be identified which can prevent the transduction of signals by the PTC protein in normal or abnormal cells.

25 The PTC protein, particularly binding fragments thereof, the gene encoding the protein, or fragments thereof, particularly fragments of at least about 18 nucleotides, frequently of at least about 30 nucleotides and up to the entire gene, more particularly sequences associated with the hydrophilic loops, may be employed in a wide variety of assays. In these situations, the particular molecules will  
30 normally be joined to another molecule, serving as a label, where the label can directly or indirectly provide a detectable signal. Various labels include radioisotopes, fluorescers, chemilumescers, enzymes, specific binding molecules,

particles, e.g. magnetic particles, and the like. Specific binding molecules include pairs, such as biotin and streptavidin, digoxin and antidigoxin etc. For the specific binding members, the complementary member would normally be labeled with a molecule which provides for detection, in accordance with known procedures. The

5 assays may be used for detecting the presence of molecules which bind to the *patched* gene or PTC protein, in isolating molecules which bind to the *patched* gene, for measuring the amount of *patched*, either as the protein or the message, for identifying molecules which may serve as agonists or antagonists, or the like.

Various formats may be used in the assays. For example, mammalian or

10 invertebrate cells may be designed where the cells respond when an agonist binds to PTC in the membrane of the cell. An expression cassette may be introduced into the cell, where the transcriptional initiation region of *patched* is joined to a marker gene, such as  $\beta$ -galactosidase, for which a substrate forming a blue dye is available. A 1.5kb fragment that responds to PTC signaling has been identified and shown to

15 regulate expression of a heterologous gene during embryonic development. When an agonist binds to the PTC protein, the cell will turn blue. By employing a competition between an agonist and a compound of interest, absence of blue color formation will indicate the presence of an antagonist. These assays are well known in the literature. Instead of cells, one may use the protein in a membrane

20 environment and determine binding affinities of compounds. The PTC may be bound to a surface and a labeled ligand for PTC employed. A number of labels have been indicated previously. The candidate compound is added with the labeled ligand in an appropriate buffered medium to the surface bound PTC. After an incubation to ensure that binding has occurred, the surface may be washed free of

25 any non-specifically bound components of the assay medium, particularly any non-specifically bound labeled ligand, and any label bound to the surface determined. Where the label is an enzyme, substrate producing a detectable product may be used. The label may be detected and measured. By using standards, the binding affinity of the candidate compound may be determined.

30 The availability of the gene and the protein allows for investigation of the development of the fetus and the role *patched* and other molecules play in such development. By employing antisense sequences of the *patched* gene, where the

sequences may be introduced in cells in culture, or a vector providing for transcription of the antisense of the *patched* gene introduced into the cells, one can investigate the role the PTC protein plays in the cellular development. By providing for the PTC protein or fragment thereof in a soluble form which can compete with  
5 the normal cellular PTC protein for ligand, one can inhibit the binding of ligands to the cellular PTC protein to see the effect of variation in concentration of ligands for the PTC protein on the cellular development of the host. Antibodies against PTC can also be used to block function, since PTC is exposed on the cell surface.

The subject gene may also be used for preparing transgenic laboratory  
10 animals, which may serve to investigate embryonic development and the role the PTC protein plays in such development. By providing for variation in the expression of the PTC protein, employing different transcriptional initiation regions which may be constitutive or inducible, one can determine the developmental effect of the differences in PTC protein levels. Alternatively, one can use the DNA to  
15 knock out the PTC protein in embryonic stem cells, so as to produce hosts with only a single functional *patched* gene or where the host lacks a functional *patched* gene. By employing homologous recombination, one can introduce a *patched* gene, which is differentially regulated, for example, is expressed to the development of the fetus, but not in the adult. One may also provide for expression of the *patched* gene in  
20 cells or tissues where it is not normally expressed or at abnormal times of development. One may provide for mis-expression or failure of expression in certain tissue to mimic a human disease. Thus, mouse models of spina bifida or abnormal motor neuron differentiation in the developing spinal cord are made available. In addition, by providing expression of PTC protein in cells in which it is  
25 otherwise not normally produced, one can induce changes in cell behavior upon binding of ligand to the PTC protein.

Areas of investigation may include the development of cancer treatments. The *wingless* gene, whose transcription is regulated in flies by PTC, is closely related to a mammalian oncogene, *Wnt-1*, a key factor in many cases of mouse  
30 breast cancer. Other Wnt family members, which are secreted signaling proteins, are implicated in many aspects of development. In flies, the signaling factor *decapentaplegic*, a member of the TGF-beta family of signaling proteins, known to

affect growth and development in mammals, is also controlled by PTC. Since members of both the TGF-beta and Wnt families are expressed in mice in places close to overlapping with *patched*, the common regulation provides an opportunity in treating cancer. Also, for repair and regeneration, proliferation competent cells making PTC protein can find use to promote regeneration and healing for damaged tissue, which tissue may be regenerated by transfecting cells of damaged tissue with the *ptc* gene and its normal transcription initiation region or a modified transcription initiation region. For example, PTC may be useful to stimulate growth of new teeth by engineering cells of the gums or other tissues where PTC protein was during an earlier developmental stage or is expressed.

Since Northern blot analysis indicates that *ptc* is present at high levels in adult lung tissue, the regulation of *ptc* expression or binding to its natural ligand may serve to inhibit proliferation of cancerous lung cells. The availability of the gene encoding PTC and the expression of the gene allows for the development of agonists and antagonists. In addition, PTC is central to the ability of neurons to differentiate early in development. The availability of the gene allows for the introduction of PTC into host diseased tissue, stimulating the fetal program of division and/or differentiation. This could be done in conjunction with other genes which provide for the ligands which regulate PTC activity or by providing for agonists other than the natural ligand.

The availability of the coding region for various *ptc* genes from various species, allows for the isolation of the 5' non-coding region comprising the promoter and enhancer associated with the *ptc* genes, so as to provide transcriptional and post-transcriptional regulation of the *ptc* gene or other genes, which allow for regulation of genes in relation to the regulation of the *ptc* gene. Since the *ptc* gene is autoregulated, activation of the *ptc* gene will result in activation of transcription of a gene under the transcriptional control of the transcriptional initiation region of the *ptc* gene. The transcriptional initiation region may be obtained from any host species and introduced into a heterologous host species, where such initiation region is functional to the desired degree in the foreign host. For example, a fragment of from about 1.5 kb upstream from the initiation codon, up to about 10kb, preferably up to about 5 kb may be used to provide for transcriptional initiation regulated by



the PTC protein, particularly the *Drosophila* 5'-non-coding region (GenBank accession no. M28418).

The following examples are offered by illustration not by way of limitation.

5

## EXPERIMENTAL

### Methods and Materials

#### I. PCR on Mosquito (*Anopheles gambiae*) Genomic DNA:

PCR primers were based on amino acid stretches of fly PTC that were not likely to diverge over evolutionary time and were of low degeneracy. Two such primers (P2R1 (SEQ ID NO:14): GGACGAATTCAARGTNCAYCARYTNTGG, P4R1: (SEQ ID NO:15) GGACGAATTCCYTCCCARAARCANTC, (the underlined sequences are Eco RI linkers) amplified an appropriately sized band from mosquito genomic DNA using the PCR. The program conditions were as follows:

15      94 °C 4 min.; 72 °C Add Taq;  
         [49 °C 30 sec.; 72 °C 90 sec.; 94 °C 15 sec] 3 times  
         [94 °C 15 sec.; 50 °C 30 sec.; 72 °C 90 sec] 35 times  
         72 °C 10 min; 4 °C hold

This band was subcloned into the EcoRV site of pBluescript II and sequenced using  
20      the USB Sequence kit.

#### II. Screen of a Butterfly cDNA Library with Mosquito PCR Product

Using the mosquito PCR product (SEQ ID NO:7) as a probe, a 3 day embryonic *Precis coenia* λgt10 cDNA library (generously provided by Sean Carroll) was screened. Filters were hybridized at 65 °C overnight in a solution containing 5xSSC, 10% dextran sulfate, 5x Denhardt's, 200 µg/ml sonicated salmon sperm DNA, and 0.5% SDS. Filters were washed in 0.1X SSC, 0.1% SDS at room temperature several times to remove nonspecific hybridization. Of the 100,000 plaques initially screened, 2 overlapping clones, L1 and L2, were isolated, which corresponded to the N terminus of butterfly PTC. Using L2 as a probe, the library filters were rescreened and 3 additional clones (L5, L7, L8) were isolated which encompassed the remainder of the *ptc* coding sequence. The full length

30

sequence of butterfly *ptc* (SEQ ID NO:3) was determined by ABI automated sequencing.

III. Screen of a *Tribolium* (beetle) Genomic Library with Mosquito PCR Product and 900 bp Fragment from the Butterfly Clone

5 A  $\lambda$ gem11 genomic library from *Tribolium castaneum* (gift of Rob Dennell) was probed with a mixture of the mosquito PCR (SEQ ID NO:7) product and BstXI/EcoRI fragment of L2. Filters were hybridized at 55 °C overnight and washed as above. Of the 75,000 plaques screened, 14 clones were identified and the  
10 SacI fragment of T8 (SEQ ID NO:1), which crosshybridized with the mosquito and butterfly probes, was subcloned into pBluescript.

IV. PCR on Mouse cDNA Using Degenerate Primers Derived from Regions Conserved in the Four Insect Homologues

15 Two degenerate PCR primers (P4REV: (SEQ ID NO:16) GGACGAATTCYTNGANTGYTTYTGGA; P22: (SEQ ID NO:17) CATACCAGCCAAGCTTGTCIGGCCARTGCAT) were designed based on a comparison of PTC amino acid sequences from fly (*Drosophila melanogaster*) (SEQ ID NO:6), mosquito (*Anopheles gambiae*) (SEQ ID NO:8), butterfly (*Precis coenia*) (SEQ ID NO:4), and beetle (*Tribolium castaneum*) (SEQ ID NO:2). I  
20 represents inosine, which can form base pairs with all four nucleotides. P22 was used to reverse transcribe RNA from 12.5 dpc mouse limb bud (gift from David Kingsley) for 90 min at 37 °C. PCR using P4REV (SEQ ID NO:17) and P22 (SEQ ID NO:18) was then performed on 1  $\mu$ l of the resultant cDNA under the following  
25 conditions:

94 °C 4 min.; 72 °C Add Taq;

[94 °C 15 sec.; 50 °C 30 sec.; 72 °C 90 sec.] 35 times

72 °C 10 min.; 4 °C hold

PCR products of the expected size were subcloned into the TA vector (Invitrogen) and sequenced with the Sequenase Version 2.0 DNA Sequencing Kit (U.S.B.).  
30

Using the cloned mouse PCR fragment as a probe, 300,000 plaques of a mouse 8.5 dpc  $\lambda$ gt10 cDNA library (a gift from Brigid Hogan) were screened at

65°C as above and washed in 2x SSC, 0.1% SDS at room temperature. 7 clones were isolated, and three (M2 M4, and M8) were subcloned into pBluescript II. 200,000 plaques of this library were rescreened using first, a 1.1 kb EcoRI fragment from M2 to identify 6 clones (M9-M16) and secondly a mixed probe containing the most N terminal (XhoI fragment from M2) and most C terminal sequences (BamHI/BglII fragment from M9) to isolate 5 clones (M17-M21). M9, M10, M14, and M17-21 were subcloned into the EcoRI site of pBluescript II (Stratagene).

V. RNA Blots and in situ Hybridizations in Whole and Sectioned Mouse Embryos

10 Northern:

A mouse embryonic Northern blot and an adult multiple tissue Northern blot (obtained from Clontech) were probed with a 900 bp EcoRI fragment from an N terminal coding region of mouse *ptc*. Hybridization was performed at 65 °C in 5x SSPE, 10x Denhardt's, 100 µg/ml sonicated salmon sperm DNA, and 2% SDS. After several short room temperature washes in 2x SSC, 0.05% SDS, the blots were washed at high stringency in 0.1X SSC, 0.1% SDS at 50C.

In situ hybridization of sections:

7.75, 8.5, 11.5, and 13.5 dpc mouse embryos were dissected in PBS and frozen in Tissue-Tek medium at -80 °C. 12-16 µm frozen sections were cut, collected onto VectaBond (Vector Laboratories) coated slides, and dried for 30-60 minutes at room temperature. After a 10 minute fixation in 4% paraformaldehyde in PBS, the slides were washed 3 times for 3 minutes in PBS, acetylated for 10 minutes in 0.25% acetic anhydride in triethanolamine, and washed three more times for 5 minutes in PBS. Prehybridization (50% formamide, 5X SSC, 250 µg/ml yeast tRNA, 500 µg/ml sonicated salmon sperm DNA, and 5x Denhardt's) was carried out for 6 hours at room temperature in 50% formamide/5x SSC humidified chambers. The probe, which consisted of 1 kb from the N-terminus of *ptc*, was added at a concentration of 200-1000 ng/ml into the same solution used for prehybridization, and then denatured for five minutes at 80 °C. Approximately 75 µl of probe were added to each slide and covered with Parafilm. The slides were incubated overnight at 65 °C in the same humidified chamber used previously. The following day, the probe was washed successively in 5X SSC (5 minutes, 65 °C),

0.2X SSC (1 hour, 65 °C), and 0.2X SSC (10 minutes, room temperature). After five minutes in buffer B1 (0.1M maleic acid, 0.15 M NaCl, pH 7.5), the slides were blocked for 1 hour at room temperature in 1% blocking reagent (Boehringer-Mannheim) in buffer B1, and then incubated for 4 hours in buffer B1 containing the DIG-AP conjugated antibody (Boehringer-Mannheim) at a 1:5000 dilution. Excess antibody was removed during two 15 minute washes in buffer B1, followed by five minutes in buffer B3 (100 mM Tris, 100mM NaCl, 5mM MgCl<sub>2</sub>, pH 9.5). The antibody was detected by adding an alkaline phosphatase substrate (350 µl 75 mg/ml X-phosphate in DMF, 450 µl 50 mg/ml NBT in 70% DMF in 100 mls of buffer B3) and allowing the reaction to proceed over-night in the dark. After a brief rinse in 10 mM Tris, 1mM EDTA, pH 8.0, the slides were mounted with Aquamount (Lerner Laboratories).

#### VI. Drosophila 5-transcriptional initiation region β-gal constructs.

A series of constructs were designed that link different regions of the *ptc* promoter from *Drosophila* to a *LacZ* reporter gene in order to study the cis regulation of the *ptc* expression pattern. See Fig. 1. A 10.8kb BamHI/BspM1 fragment comprising the 5'-non-coding region of the mRNA at its 3'-terminus was obtained and truncated by restriction enzyme digestion as shown in Fig. 1. These expression cassettes were introduced into *Drosophila* lines using a P-element vector (Thummel et al., Gene 74, 445-456 (1988), which were injected into embryos, providing flies which could be grown to produce embryos. (See Spradling and Rubin, Science (1982) 218, 341-347 for a description of the procedure.) The vector used a pUC8 background into which was introduced the white gene to provide for yellow eyes, portions of the P-element for integration, and the constructs were inserted into a polylinker upstream from the *LacZ* gene. The resulting embryos were stained using antibodies to LacZ protein conjugated to HRP and the embryos developed with OPD dye to identify the expression of the *LacZ* gene. The staining pattern is described in Fig. 1, indicating whether there was staining during the early and late development of the embryo.

#### VII. Isolation of a Mouse *ptc* Gene

Homologues of fly PTC (SEQ ID NO:6) were isolated from three insects: mosquito, butterfly and beetle, using either PCR or low stringency library screens. PCR primers to six amino acid stretches of PTC of low mutatability and degeneracy were designed. One primer pair, P2 and P4, amplified an homologous fragment of

5 *ptc* from mosquito genomic DNA that corresponded to the first hydrophilic loop of the protein. The 345bp PCR product (SEQ ID NO:7) was subcloned and sequenced and when aligned to fly PTC, showed 67% amino acid identity.

The cloned mosquito fragment was used to screen a butterfly  $\lambda$ GT 10 cDNA library. Of 100,000 plaques screened, five overlapping clones were isolated and

10 used to obtain the full length coding sequence. The butterfly PTC homologue (SEQ ID NO:4) is 1,311 amino acids long and overall has 50% amino acid identity (72% similarity) to fly PTC. With the exception of a divergent C-terminus, this homology is evenly spread across the coding sequence. The mosquito PCR clone (SEQ ID NO:7) and a corresponding fragment of butterfly cDNA were used to screen a beetle

15  $\lambda$ gem11 genomic library. Of the plaques screened, 14 clones were identified. A fragment of one clone (T8), which hybridized with the original probes, was subcloned and sequenced. This 3kb piece contains an 89 amino acid exon (SEQ ID NO:2) which is 44% and 51% identical to the corresponding regions of fly and butterfly PTC respectively.

20 Using an alignment of the four insect homologues in the first hydrophilic loop of the PTC, two PCR primers were designed to a five and six amino acid stretch which were identical and of low degeneracy. These primers were used to isolate the mouse homologue using RT-PCR on embryonic limb bud RNA. An appropriately sized band was amplified and upon cloning and sequencing, it was found to encode a

25 protein 65% identical to fly PTC. Using the cloned PCR product and subsequently, fragments of mouse *ptc* cDNA, a mouse embryonic  $\lambda$ cDNA library was screened. From about 300,000 plaques, 17 clones were identified and of these, 7 form overlapping cDNA's which comprise most of the protein-coding sequence (SEQ ID NO:9).

30

VIIa. Developmental and Tissue Distribution of Mouse PTC RNA

In both the embryonic and adult Northern blots, the *ptc* probe detects a single 8kb message. Further exposure does not reveal any additional minor bands. Developmentally, *ptc* mRNA is present in low levels as early as 7 dpc and becomes quite abundant by 11 and 15 dpc. While the gene is still present at 17 dpc, the Northern blot indicates a clear decrease in the amount of message at this stage. In the adult, *ptc* RNA is present in high amounts in the brain and lung, as well as in moderate amounts in the kidney and liver. Weak signals are detected in heart, spleen, skeletal muscle, and testes.

#### 10 VIIb. In situ Hybridization of Mouse PTC in Whole and Section Embryos

Northern analysis indicates that *ptc* mRNA is present at 7 dpc, while there is no detectable signal in sections from 7.75 dpc embryos. This discrepancy is explained by the low level of transcription. In contrast, *ptc* is present at high levels along the neural axis of 8.5 dpc embryos. By 11.5 dpc, *ptc* can be detected in the developing lung buds and gut, consistent with its adult Northern profile. In addition, the gene is present at high levels in the ventricular zone of the central nervous system, as well as in the zona limitans of the prosencephalon. *ptc* is also strongly transcribed in the condensing cartilage of 11.5 and 13.5 dpc limb buds, as well as in the ventral portion of the somites, a region which is prospective sclerotome and eventually forms bone in the vertebral column. *ptc* is present in a wide range of tissues from endodermal, mesodermal and ectodermal origin supporting its fundamental role in embryonic development.

#### VIII. Isolation of the Human *ptc* Gene

25 To isolate human *ptc* (hptc),  $2 \times 10^5$  plaques from a human lung cDNA library (HL3022a, Clontech) were screened with a 1kbp mouse *ptc* fragment, M2-2. Filters were hybridized overnight at reduced stringency (60 °C in 5X SSC, 10% dextran sulfate, 5X Denhardt's, 0.2 mg/ml sonicated salmon sperm DNA, and 0.5% SDS). Two positive plaques (H1 and H2) were isolated, the inserts cloned into pBluescript, and upon sequencing, both contained sequence highly similar to the mouse *ptc* homolog. To isolate the 5' end, an additional  $6 \times 10^5$  plaques were screened in duplicate with M2-3 EcoR I and M2-3 Xho I (containing 5' untranslated

sequence of mouse *ptc*) probes. Ten plaques were purified and of these, 6 inserts were subcloned into pBluescript. To obtain the full coding sequence, H2 was fully and H14, H20, and H21 were partially sequenced. The 5.1kbp of human *ptc* sequence (SEQ ID NO:18) contains an open reading frame of 1447 amino acids  
 5 (SEQ ID NO:19) that is 96% identical and 98% similar to mouse *ptc*. The 5' and 3' untranslated sequences of human *ptc* (SEQ ID NO:18) are also highly similar to mouse *ptc* (SEQ ID NO:09) suggesting conserved regulatory sequence.

#### IX. Comparison of Mouse, Human, Fly and Butterfly Sequences

10 The deduced mouse PTC protein sequence (SEQ ID NO:10) has about 38% identical amino acids to fly PTC over about 1,200 amino acids. This amount of conservation is dispersed through much of the protein excepting the C-terminal region. The mouse protein also has a 50 amino acid insert relative to the fly  
 15 of *hedgehog* between fly and mouse, one concludes that *ptc* functions similarly in the two organisms. A comparison of the amino acid sequences of mouse (mptc) (SEQ ID NO:10), human (hptc) (SEQ ID NO:19), butterfly (bptc)(SEQ ID NO:4) and drosophila (*ptc*) (SEQ ID NO:6) is shown in Table 1.

**TABLE 1**

20	alignment of human, mouse, fly, and butterfly PTC homologs	
	alignment of human, mouse, fly, and butterfly ptc homologs	
25	HPTC	MASAGNAAEPQDR--GGGGSGCIGAPGRPAGGGRRRRRTGGLRRAAAPDRDYLHRPSYCDA
	MPTC	MASAGNAA-----GALGRQAGGGRRRRRTGGPHRA-APDRDYLHRPSYCDA
	PTC	M-----DRDSLPRVPDTHGD--VVDE-----KLFSDL-----YI-RTSWVDA
30	BPTC	MVAPDSEAPSNPRITA AHESPCATEA-----RHSADL-----YI-RTSWVDA
		* . . . . . * . . . . . *
	HPTC	AFALEQISKGKATGRKAPLWLRKFQRLLFKLGCIYIQKNCGKFLVVGLLIFGAFVAVGLKA
	MPTC	AFALEQISKGKATGRKAPLWLRKFQRLLFKLGCIYIQKNCGKFLVVGLLIFGAFVAVGLKA
35	PTC	QVALDQIDKGKARGSRTAIYLRVFSHLETLGSSVQKHAGKVLVFAILVLSTFCVGLKS
	BPTC	ALALSELEKGNIEGGRTSLWIRAWLQEQLFILGCFLOGDAGKVLVFAILVLSTFCVGLKS
		** . . . . . * . . . . . * . . . . . * . . . . . *
	HPTC	ANLETNVEELWVEVGGRVSRELNYTRQKIGEEAMFNPQLMIQTPKEEGANVLTTEALLQH
40	MPTC	ANLETNVEELWVEVGGRVSRELNYTRQKIGEEAMFNPQLMIQTPKEEGANVLTTEALLQH
	PTC	AQIHQKVHQLWVQEGGRLEAEIAYTQKTIGEDSATHQLLIQTTHDPNASVLHPQALLAH
	BPTC	AQIHTRVDQLWVQEGGRLEAEIAYTQKTIGEDSATHQLLIQTTHDPNASVLHPQALLAH
		* . . . . . * . . . . . * . . . . . * . . . . . *
	HPTC	LDSALQASRVHVYMYNRQWKLEHLCKYKSGELITET-GYMDQII EYLYPCLIIITPLDCFE

WO 96/11260

MPTC LDSALQASRVHVYMYNRQWKLEHLICYKSGELITET-GYMDQIIEYLYPCLIIITPLDCFEW  
 PTC LEVLVKATAVKVHLYDTEWGLRDMCNMPSTPSFEGIIYIEQILRHLIPCSIITPLDCFEW  
 BPTC LKVVHAATRVTVHMYDIEWRLKDLCSPSIPDFEGYHHIESIIDNVIPCAIITPLDCFEW  
 \* \* \* \* \*

5 HPTC GAKLQSGTAYLLGKPPLR-----WTNFDPLEFLEELK-----KINYQVDSWEEMLNKAEV  
 MPTC GAKLQSGTAYLLGKPPLR-----WTNFDPLEFLEELK-----KINYQVDSWEEMLNKAEV  
 PTC GSQLL-GPESAVVIPGLNQRLLTTLNPASVMQYMKQKMSSEEKISDFETVEQYMKRAAI  
 BPTC GSKLL-GPDYPIYVPHLKHKLQWTHLNPLEVVEEVK-KL---KFQFPLSTIEAYMKRAGI  
 \* \* \* \* \*

10 HPTC GHGYMDRCPCLNPADPDCPATAPNKNSTKPLDMALVLNGGCHGLSRKYMHWQEELIVGGTV  
 MPTC GHGYMDRCPCLNPADPDCPATAPNKNSTKPLDVALVLNGGCQGLSRKYMHWQEELIVGGTV  
 PTC GSGYMEKPCLNPLNPNCPDTPAPNKNSTQPPDVGAILSGGCYGYAAKHMHWPEELIVGGRK  
 BPTC TSAYMKKPCLDPTDPHCPATAPNKKSGHIPDVAELSHGCGYFAAAYMHWPEQLIVGGAT  
 \* \* \* \* \*

15 HPTC KNSTGKLVSAAHALQTMFQMTPKQMYEHFKGYEYVSHINWNEKAAAILAWQRTYVEVV  
 MPTC KNATGKLVSAAHALQTMFQMTPKQMYEHFRGYDYVSHINWNEKAAAILAWQRTYVEVV  
 PTC RNRSGHLRKAQALQSVVQLMTEKEMYDQWQDNKYVHHLGWTQEKAAEVLNAWQRNFSREV  
 BPTC RNSTSLARSARALQTVVQLMGEREMYEWADHYKVHQIGWNQEKAAAVLDAWQRKFAAEV  
 \* \* \* \* \*

20 HPTC HQSVAQNSTQK-----VLSFTTTTLDLILKSFSFSDSVIRVASGYLLMLAYACLTMLRW-DC  
 MPTC HQSVAQNSTQK-----VLPFTTTTLDLILKSFSFSDSVIRVASGYLLMLAYACLTMLRW-DC  
 PTC EQLLRKQSRATNYDIYVFSSAALDDILAKFSHPALSIVIGVAVTVLYAFCTLLRWRDP  
 BPTC RKI-TTSGSVSSAYSFPSTSTLNDILGKFSEVSLKNIILGYMFMLIYVAVTLIQWRDP  
 \* \* \* \* \*

25 HPTC SKSQGAVGLAGVLLVALSVAAGLGLCSLIGISFNAATTQVLPFLALGVGVDDVFLLAHAF  
 MPTC SKSQGAVGLAGVLLVALSVAAGLGLCSLIGISFNAATTQVLPFLALGVGVDDVFLLAHAF  
 PTC VRGQSSVGAVGVLMLCFSTAAGLGLSALLGIVFNAASTQVVPFLALGLGVDDHIFMLTAAY  
 BPTC IRSQAGVGIAGVLLSITVAAGLGFALLGIPFNASTQIVPFLALGLGVQDMFLHTY  
 \* \* \* \* \*

30 HPTC SETGQNKRI PFEDRTGECLKRTGASVALTSISNVTAFFMAALIPIPALRAFSLQAQAVVV  
 MPTC SETGQNKRI PFEDRTGECLKRTGASVALTSISNVTAFFMAALIPIPALRAFSLQAQAVVV  
 PTC AESN-----RREQTKLILKKVGPSILFSACSTAGSFFAAAFIPVPAKVFCLQAIVMC  
 BPTC VEQAGD--VPREERTGLVLKKSGLSVLLASLCNVMAFLAAALLPIPAFRVFCLOQAAILL  
 \* \* \* \* \*

35 HPTC FNFAMVLLIFPAILSMDLRREDRRLDIFCCFTSPCVSRVIOVEPQAYTDTHDNTRYSP  
 MPTC FNFAMVLLIFPAILSMDLRREDRRLDIFCCFTSPCVSRVIOVEPQAYTEPHSNTRYSP  
 PTC SNLAAALLVFPAMISLDLRRRTAGRADIFCCCF-PVWKEQPKVAPPVLPNNNGR----  
 BPTC FNLGSILLVFPAMISLDLRRRSARADLLCLM-P---ESP-----LPKKKI PER----  
 \* \* \* \* \*

40 HPTC PPYSSHSFAHETQITMQSTVQLRTEYDPHTHVYYTTAEPRSEISVQPVTVTQDT LSCQSP  
 MPTC PPYTSHSFAHETHITMQSTVQLRTEYDPHTHVYYTTAEPRSEISVQPVTVTQDNLSQSP  
 PTC -----GARHPKSCNNNRVPLPAQNPLLEQRA  
 BPTC -----AKTRKNDKTHRID-TTROPLDPDVS  
 \* \* \* \* \*

45 HPTC ESTSSTRDLLSQFSDSSLHCLEPPCTKWTLSSFAEKHYAPFLLKPKAKVVVIFLFLGLLG  
 MPTC ESTSSTRDLLSQFSDSSLHCLEPPCTKWTLSSFAEKHYAPFLLKPKAKVVVILFLGLLG  
 PTC DIPGSS-----HSLASF-----SLATFAFQHYTFPLMRSVKFLTVMGFLAALI  
 BPTC ENVTKT-----CCL-SV-----SLTKWAKNQYAPFIMRPAVKVTSMLALIAVIL  
 \* \* \* \* \*

50 HPTC VSLYGTTRVRDGLDLTDIVPRETREYDFIAAQFKYFSFYNYIVTOKA-DYPNIQHLLYD  
 MPTC VSLYGTTRVRDGLDLTDIVPRETREYDFIAAQFKYFSFYNYIVTOKA-DYPNIQHLLYD  
 PTC SSLYASTRLQDGLDIIDLVPKDSNEHKFLDAQTRLFYGFYSMYAVTQGNFEYPTQQLLRD  
 BPTC TSVWGATKVKDGLDLTDIVPENTDEHEFLSRQEKYFGFYNYAVTQGNFEYPTNQKLLYE  
 \* \* \* \* \*

55 HPTC  
 MPTC  
 PTC  
 BPTC

60 HPTC  
 MPTC  
 PTC  
 BPTC

65



[illegible]

The identity of ten other clones recovered from the mouse library is not determined. These cDNAs cross-hybridize with mouse *ptc* sequence, while differing as to their restriction maps. These genes encode a family of proteins related to the  
5 *patched* protein. Alignment of the human and mouse nucleotide sequences, which includes coding and noncoding sequence, reveals 89% identity.

In accordance with the subject invention, mammalian *patched* genes, including the mouse and human genes, are provided which allow for high level production of  
10 the *patched* protein, which can serve many purposes. The *patched* protein may be used in a screening for agonists and antagonists, for isolation of its ligand, particularly *hedgehog*, more particularly *Sonic hedgehog*, and for assaying for the transcription of the mRNA *ptc*. The protein or fragments thereof may be used to produce antibodies specific for the protein or specific epitopes of the protein. In  
15 addition, the gene may be employed for investigating embryonic development, by screening fetal tissue, preparing transgenic animals to serve as models, and the like.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were  
20 specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention  
25 that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(i) APPLICANT: THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY

(ii) TITLE OF INVENTION: Patched Genes and their Use

(iii) NUMBER OF SEQUENCES: 19

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(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: PCT/US95/  
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(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Rowland, Bertram I  
(B) REGISTRATION NUMBER: 20015  
(C) REFERENCE/DOCKET NUMBER: a60190-1

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 415-781-1989  
(B) TELEFAX: 415-398-3249

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 736 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AACNNC NNTN NATGGCACCC CCNCCCAACC TTTNNCCNN NTAANCAAAA NNCCCCNTTT

60

NATACCCCT NTAANANTTT TCCACCNNNC NNAAANNCCN CTGNANACNA NGNAAANCCN 120  
 TTTTNAACC CCCCCACCC GGAATTCCNA NTNCCNCCC CCAAATTACA ACTCCAGNCC 180  
 AAAATTNANA NAATTGGTCC TAACCTAACC NATNGTTGTT ACGGTTTCCC CCCCCAAATA 240  
 CATGCACTGG CCCGAACACT TGATCGTTGC CGTTCCAATA AGAATAAATC TGGTCATATT 300  
 AAACAAGCCN AAAGCTTTAC AAAGTGTGT ACAATTAATG GGCGAACACG AACTGTTCTGA 360  
 ATTCTGGTCT GGACATTACA AAGTGCACCA CATCGGATGG AACCAGGAGA AGGCCACAAC 420  
 CGTACTGAAC GCCTGGCAGA AGAAGTTCGC ACAGGTTGGT GGTGGCGCA AGGAGTAGAG 480  
 TGAATGGTGG TAATTTTGG TTGTTCCAGG AGGTGGATCG TCTGACGAAG AGCAAGAAGT 540  
 CGTCGAATTA CATCTTCGTG ACGTTCTCCA CCGCCAATTT GAACAAGATG TTGAAGGAGG 600  
 CGTCGAANAC GGACGTGGTG AAGCTGGGGG TGGTGCTGGG GGTGGCGCG GTGTACGGGT 660  
 GGGTGGCCCA GTCGGGGCTG GCTGCCTTGG GAGTGCTGGT CTTNGCGNGC TNCNATTCCG 720  
 CCTATAGTNA GNCGTA 736

## (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 107 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Xaa Pro Pro Pro Asn Tyr Asn Ser Xaa Pro Lys Xaa Xaa Xaa Leu Val  
 1 5 10 15  
 Leu Thr Pro Xaa Val Val Thr Val Ser Pro Pro Lys Tyr Met His Trp  
 20 25 30  
 Pro Glu His Leu Ile Val Ala Val Pro Ile Arg Ile Asn Leu Val Ile  
 35 40 45  
 Leu Asn Lys Pro Lys Ala Leu Gln Thr Val Val Gln Leu Met Gly Glu  
 50 55 60  
 His Glu Leu Phe Glu Phe Trp Ser Gly His Tyr Lys Val His His Ile  
 65 70 75 80  
 Gly Trp Asn Gln Glu Lys Ala Thr Thr Val Leu Asn Ala Trp Gln Lys  
 85 90 95  
 Lys Phe Ala Gln Val Gly Gly Trp Arg Lys Glu

100

105

## (2) INFORMATION FOR SEQ ID NO:3:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5187 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (11) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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GGGTCTGTCA CCCGGAGCCG GAGTCCCCGG CGGCCAGCAG CGTCCTCGOG AGCCGAGCGC      60
CCAGGCGCGC CCGGAGCCCG CGGCGGCGGC GGCAACATGG CCTCGGCTGG TAACGCGGCC      120
GGGGCCCTGG GCAGGCAGGC CGGCGGCGGG AGGCGCAGAC GGACCGGGGG ACCGCACCGC      180
GCCGCGCCGG ACCGGGACTA TCTGCACCGG CCCAGCTACT GCGACCGCCG CTTGCTCTG      240
GAGCAGATT CCAAGGGGAA GGCTACTGGC CGGAAAGCGC CGCTGTGGCT GAGAGCGAAG      300
TTTCAGAGAC TCTTATTTAA ACTGGGTTGT TACATTCAA AGAACTGCGG CAAGTTTTTG      360
GTTGTGGGTC TCCTCATATT TGGGGCCTTC GCTGTGGGAT TAAAGGCAGC TAATCTCGAG      420
ACCAACGTGG AGGAGCTGTG GGTGGAAGTT GGTGGACGAG TGAGTCGAGA ATTAAATTAT      480
ACCCGTCAGA AGATAGGAGA AGAGGCTATG TTTAATCCTC AACTCATGAT ACAGACTCCA      540
AAAGAAGAAG GCGCTAATGT TCTGACCACA GAGGCTCTCC TGCAACACCT GGA CTAGCA      600
CTCCAGGCCA GTCGTGTGCA CGTCTACATG TATAACAGGC AATGGAAGTT GGAACATTTG      660
TGCTACAAAT CAGGGGAACT TATCAGGAG ACAGGTTACA TGGATCAGAT AATAGAATAC      720
CTTTACCCTT GCTTAATCAT TACACCTTTG GACTGCTTCT GCGAAGGGGC AAAGCTACAG      780
TCCGGGACAG CATACTCCT AGGTAAGCCT CCTTTACGGT GGACAAACTT TGACCCCTTG      840
GAATTCCTAG AAGAGTTAAA GAAAATAAAC TACCAAGTGG ACAGCTGGGA GGAAATGCTG      900
AATAAGCCG AAGTTGGCCA TGGGTACATG GACCGGCCTT GCCTCAACCC AGCCGACCCA      960
GATTGCCCTG CCACAGCCCC TAACAAAAAT TCAACCAAAC CTCTTGATGT GGCCCTTGTT     1020
TTGAATGGTG GATGTCAAGG TTTATCCAGG AAGTATATGC ATTGGCAGGA GGAGTTGATT     1080
GTGGGTGGTA CCGTCAAGAA TGCCACTGGA AAAGTTGTCA GCGCTCACGC CCTGCAAACC     1140
ATGTTCCAGT TAATGACTCC CAAGCAAATG TATGAACACT TCAGGGGCTA CGACTATGTC     1200
TCTCACATCA ACTGGAATGA AGACAGGGCA GCCGCCATCC TGGAGGCCTG GCAGAGGACT     1260

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TACGTGGAGG TGGTTCATCA AAGTGTCGCC CCAAACCTCCA CTCAAAAGGT GCTTCCCTTC	1320
ACAACCACGA CCCTGGACGA CATCCTAAAA TCCTTCTCTG ATGTCAGTGT CATCCGAGTG	1380
GCCAGCGGCT ACCTACTGAT GCTTGCCTAT GCCTGTTTAA CCATGCTGCG CTGGGACTGC	1440
TCCAAGTCCC AGGGTGCCGT GGGGCTGGCT GGCGTCCTGT TGGTTGCGCT GTCAGTGGCT	1500
GCAGGATTGG GCCTCTGCTC CTTGATTGGC ATTTCTTTTA ATGCTGCGAC AACTCAGGTT	1560
TTGCCGTTTC TTGCTCTTGG TGTGGTGTG GATGATGTCT TCCTCCTGGC CCATGCATTC	1620
AGTGAAACAG GACAGAATAA GAGGATTCCA TTTGAGGACA GGAAGTGGGA GTGCCTCAAG	1680
CGCACCGGAG CCAGCGTGGC CCTCACCTCC ATCAGCAATG TCACCGCCTT CTTTCATGGCC	1740
GCATTGATCC CTATCCCTGC CCTGCGAGCG TTCTCCCTCC AGGCTGCTGT GGTGGTGGTA	1800
TTCAATTTTG CTATGGTTCT GCTCATTITT CCTGCAATTC TCAGCATGGA TTTATACAGA	1860
CGTGAGGACA GAAGATTGGA TATTTTCTGC TGTTTCACAA GCCCCTGTGT CAGCAGGGTG	1920
ATTCAAGTTG AGCCACAGGC CTACACAGAG CCTCACAGTA ACACCCGGTA CAGCCCCCA	1980
CCCCCATACA CCAGCCACAG CTTGCCCCAC GAAACCCATA TCACTATGCA GTCCACCGTT	2040
CAGCTCCGCA CAGAGTATGA CCCTCACACG CACGTGTACT ACACCACCGC CGAGCCACGC	2100
TCTGAGATCT CTGTACAGCC TGTTACCGTC ACCCAGGACA ACCTCAGCTG TCAGAGTCCC	2160
GAGAGCACCA GCTCTACCAG GGACCTGCTC TCCCAGTTCT CAGACTCCAG CCTCCACTGC	2220
CTCGAGCCCC CCTGCACCAA GTGGACACTC TCTTCGTTTG CAGAGAAGCA CTATGCTCCT	2280
TTCTCTCTGA AACCCAAAGC CAAGGTTGTG GTAATCCTTC TTTCTCTGGG CTTGCTGGGG	2340
GTCAGCCTTT ATGGGACCAC CCGAGTGAGA GACGGGCTGG ACCTCACGGA CATTGTTCCC	2400
CGGGAAACCA GAGAATATGA CTTCATAGCT GCCCAGTTCA AGTACTTCTC TTTCTACAAC	2460
ATGTATATAG TCACCCAGAA AGCAGACTAC CCGAATATCC AGCACCTACT TTACGACCTT	2520
CATAAGAGTT TCAGCAATGT GAAGTATGTC ATGCTGGAGG AGAACAAGCA ACTTCCCCAA	2580
ATGTGGCTGC ACTACTTTAG AGACTGGCTT CAAGGACTTC AGGATGCATT TGACAGTGAC	2640
TGGGAAACTG GGAGGATCAT GCCAAACAAT TATAAAAATG GATCAGATGA CGGGGTCCTC	2700
GCTTACAAAC TCCTGGTGCA GACTGGCAGC CGAGACAAGC CCATCGACAT TAGTCAGTTG	2760
ACTAAACAGC GTCTGGTAGA CGCAGATGGC ATCATTAAATC CGAGCGCTTT CTACATCTAC	2820
CTGACCGCTT GGGTCAGCAA CGACCCTGTA GCTTACGCTG CCTCCCAGGC CAACATCCGG	2880
CCTCACCGGC CGGAGTGGGT CCATGACAAA GCCGACTACA TGCCAGAGAC CAGGCTGAGA	2940
ATCCCAGCAG CAGAGCCCAT CGAGTACGCT CAGTTCCCTT TCTACCTCAA CGGCCTACGA	3000

GACACCTCAG	ACTTTGTGGA	AGCCATAGAA	AAAGTGAGAG	TCATCTGTAA	CAACTATACG	3060
AGCCTGGGAC	TGTCCAGCTA	CCCCAATGGC	TACCCCTTCC	TGTTCTGGGA	GCAATACATC	3120
AGCCTGCGCC	ACTGGCTGCT	GCTATCCATC	AGCGTGGTGC	TGGCCTGCAC	GTTTCTAGTG	3180
TGCGCAGTCT	TCCTCCTGAA	CCCCTGGACG	GCCGGGATCA	TTGTCATGGT	CCTGGCTCTG	3240
ATGACCGTTG	AGCTCTTTGG	CATGATGGGC	CTCATTGGGA	TCAAGCTGAG	TGCTGTGCCT	3300
TGTTGTCATC	TGATTGCATC	TGTTGGCATC	GGAGTGGAGT	TCACCGTCCA	CGTGGCTTTG	3360
GCCTTTCTGA	CAGCCATTGG	GGACAAGAAC	CACAGGGCTA	TGCTCGCTCT	GGAACACATG	3420
TTTGCTCCCG	TTCTGGACGG	TGCTGTGTCC	ACTCTGCTGG	GTGTACTGAT	GCTTGCAAGG	3480
TCCGAATTTG	ATTTCAATTG	CAGATACTTC	TTTGCCGTCC	TGGCCATTCT	CACCGTCTTG	3540
GGGGTTCTCA	ATGGACTGGT	TCTGCTGCCT	GTCTCTTAT	CCTTCTTTGG	ACCGTGTCTT	3600
GAGGTGTCTC	CAGCCAATGG	CCTAAACOGA	CTGCCCACTC	CTTCGCCTGA	GCCGCCTCCA	3660
AGTGTCGTCC	GGTTTGCCGT	GCCTCCTGGT	CACACGAACA	ATGGGTCTGA	TTCCTCCGAC	3720
TCGGAGTACA	GCTCTCAGAC	CACGGTGTCT	GGCATCAGTG	AGGAGCTCAG	GCAATACGAA	3780
GCACAGCAGG	GTGCCGGAGG	CCCTGCCCAC	CAAGTGATTG	TGGAAGCCAC	AGAAAACCCCT	3840
GTCTTTGCCC	GGTCCACTGT	GGTCCATCCG	GACTCCAGAC	ATCAGCCTCC	CTTGACCCCT	3900
CGGCAACAGC	CCCACCTGGA	CTCTGGCTCC	TTGTCCCCTG	GACGGCAAGG	CCAGCAGCCT	3960
CCAAGGGATC	CCCCTAGAGA	AGGCTTGCGG	CCACCCCCCT	ACAGACCGCG	CAGAGACGCT	4020
TTTGAAATTT	CTACTGAAGG	GCATTCTGGC	CCTAGCAATA	GGGACCGCTC	AGGGCCCCGT	4080
GGGGCCCCGT	CTCACAACCC	TCGGAACCCA	ACGTCCACCG	CCATGGGCAG	CTCTGTGCCC	4140
AGCTACTGCC	AGCCCATCAC	CACTGTGACG	GCTTCTGCTT	CGGTGACTGT	TGCTGTGCAT	4200
CCCCCGCCTG	GACCTGGGCG	CAACCCCCGA	GGGGGGCCCT	GTCCAGGCTA	TGAGAGCTAC	4260
CCTGAGACTG	ATCACGGGGT	ATTTGAGGAT	CCTCATGTGC	CTTTTCATGT	CAGGTGTGAG	4320
AGGAGGGACT	CAAAGGTGGA	GGTCATAGAG	CTACAGGACG	TGGAATGTGA	GGAGAGGCCG	4380
TGGGGGAGCA	GCTCCAACCT	AGGGTAATTA	AAATCTGAAG	CAAAGAGGCC	AAAGATTGGA	4440
AAGCCCCGCC	CCCACCTCTT	TCCAGAACTG	CTTGAAGAGA	ACTGCTTGGA	ATTATGGGAA	4500
GGCAGTTCAT	TGTTACTGTA	ACTGATTGTA	TTATTKKGTG	AAATATTTCT	ATAAATATTT	4560
AARAGGTGTA	CACATGTAAT	ATACATGGAA	ATGCTGTACA	GTCTATTTCC	TGGGGCCTCT	4620
CCACTCCTGC	CCCAGAGTGG	GGAGACCACA	GGGGCCCTTT	CCCCTGTGTA	CATTGGTCTC	4680
TGTGCCACAA	CCAAGCTTAA	CTTAGTTTAA	AAAAAATCT	CCCAGCATAT	GTGCTGTCTG	4740

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CTTAAATATT GTATAATTTA CTTGTATAAT TCTATGCAAA TATTGCTTAT GTAATAGGAT 4800  
 TATTTGTAAA GGTTCCTGTT TAAAATATTT TAAATTTGCA TATCACAACC CTGTGGTAGG 4860  
 ATGAATTGTT ACTGTTAACT TTTGAACACG CTATGCCGTGG TAATTGTTTA ACGAGCAGAC 4920  
 ATGAAGAAAA CAGGTTAATC CCAGTGGCTT CTCTAGGGGT AGTTGTATAT GGTTCGCATC 4980  
 GGTGGATGTG TGTGTGCATG TGA CTTTCCA ATGTACTGTA TTGTGGTTTG TTGTTGTTGT 5040  
 TGCTGTTGTT GTTCATTTTG GTGTTTTTGG TTGCTTTGTA TGATCTTAGC TCTGGCCTAG 5100  
 GTGGGCTGGG AAGGTCCAGG TCTTTTTCTG TCGTGATGCT GGTGGAAAGG TGACCCCAAT 5160  
 CATCTGTCCT ATTCTCTGGG ACTATTC 5187

## (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1311 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Val Ala Pro Asp Ser Glu Ala Pro Ser Asn Pro Arg Ile Thr Ala  
 1 5 10 15  
 Ala His Glu Ser Pro Cys Ala Thr Glu Ala Arg His Ser Ala Asp Leu  
 20 25 30  
 Tyr Ile Arg Thr Ser Trp Val Asp Ala Ala Leu Ala Leu Ser Glu Leu  
 35 40 45  
 Glu Lys Gly Asn Ile Glu Gly Gly Arg Thr Ser Leu Trp Ile Arg Ala  
 50 55 60  
 Trp Leu Gln Glu Gln Leu Phe Ile Leu Gly Cys Phe Leu Gln Gly Asp  
 65 70 75 80  
 Ala Gly Lys Val Leu Phe Val Ala Ile Leu Val Leu Ser Thr Phe Cys  
 85 90 95  
 Val Gly Leu Lys Ser Ala Gln Ile His Thr Arg Val Asp Gln Leu Trp  
 100 105 110  
 Val Gln Glu Gly Gly Arg Leu Glu Ala Glu Leu Lys Tyr Thr Ala Gln  
 115 120 125  
 Ala Leu Gly Glu Ala Asp Ser Ser Thr His Gln Leu Val Ile Gln Thr  
 130 135 140



Ala Lys Asp Pro Asp Val Ser Leu Leu His Pro Gly Ala Leu Leu Glu  
 145 150 155 160  
 His Leu Lys Val Val His Ala Ala Thr Arg Val Thr Val His Met Tyr  
 165 170 175  
 Asp Ile Glu Trp Arg Leu Lys Asp Leu Cys Tyr Ser Pro Ser Ile Pro  
 180 185 190  
 Asp Phe Glu Gly Tyr His His Ile Glu Ser Ile Ile Asp Asn Val Ile  
 195 200 205  
 Pro Cys Ala Ile Ile Thr Pro Leu Asp Cys Phe Trp Glu Gly Ser Lys  
 210 215 220  
 Leu Leu Gly Pro Asp Tyr Pro Ile Tyr Val Pro His Leu Lys His Lys  
 225 230 235 240  
 Leu Gln Trp Thr His Leu Asn Pro Leu Glu Val Val Glu Glu Val Lys  
 245 250 255  
 Lys Leu Lys Phe Gln Phe Pro Leu Ser Thr Ile Glu Ala Tyr Met Lys  
 260 265 270  
 Arg Ala Gly Ile Thr Ser Ala Tyr Met Lys Lys Pro Cys Leu Asp Pro  
 275 280 285  
 Thr Asp Pro His Cys Pro Ala Thr Ala Pro Asn Lys Lys Ser Gly His  
 290 295 300  
 Ile Pro Asp Val Ala Ala Glu Leu Ser His Gly Cys Tyr Gly Phe Ala  
 305 310 315 320  
 Ala Ala Tyr Met His Trp Pro Glu Gln Leu Ile Val Gly Gly Ala Thr  
 325 330 335  
 Arg Asn Ser Thr Ser Ala Leu Arg Lys Ala Arg Xaa Leu Gln Thr Val  
 340 345 350  
 Val Gln Leu Met Gly Glu Arg Glu Met Tyr Glu Tyr Trp Ala Asp His  
 355 360 365  
 Tyr Lys Val His Gln Ile Gly Trp Asn Gln Glu Lys Ala Ala Ala Val  
 370 375 380  
 Leu Asp Ala Trp Gln Arg Lys Phe Ala Ala Glu Val Arg Lys Ile Thr  
 385 390 395 400  
 Thr Ser Gly Ser Val Ser Ser Ala Tyr Ser Phe Tyr Pro Phe Ser Thr  
 405 410 415  
 Ser Thr Leu Asn Asp Ile Leu Gly Lys Phe Ser Glu Val Ser Leu Lys  
 420 425 430  
 Asn Ile Ile Leu Gly Tyr Met Phe Met Leu Ile Tyr Val Ala Val Thr  
 435 440 445  
 Leu Ile Gln Trp Arg Asp Pro Ile Arg Ser Gln Ala Gly Val Gly Ile

450                      455                      460  
 Ala Gly Val Leu Leu Leu Ser Ile Thr Val Ala Ala Gly Leu Gly Phe  
 465                      470                      475                      480  
 Cys Ala Leu Leu Gly Ile Pro Phe Asn Ala Ser Ser Thr Gln Ile Val  
                     485                      490                      495  
 Pro Phe Leu Ala Leu Gly Leu Gly Val Gln Asp Met Phe Leu Leu Thr  
                     500                      505                      510  
 His Thr Tyr Val Glu Gln Ala Gly Asp Val Pro Arg Glu Glu Arg Thr  
                     515                      520                      525  
 Gly Leu Val Leu Lys Lys Ser Gly Leu Ser Val Leu Leu Ala Ser Leu  
                     530                      535                      540  
 Cys Asn Val Met Ala Phe Leu Ala Ala Ala Leu Leu Pro Ile Pro Ala  
 545                      550                      555                      560  
 Phe Arg Val Phe Cys Leu Gln Ala Ala Ile Leu Leu Leu Phe Asn Leu  
                     565                      570                      575  
 Gly Ser Ile Leu Leu Val Phe Pro Ala Met Ile Ser Leu Asp Leu Arg  
                     580                      585                      590  
 Arg Arg Ser Ala Ala Arg Ala Asp Leu Leu Cys Cys Leu Met Pro Glu  
                     595                      600                      605  
 Ser Pro Leu Pro Lys Lys Lys Ile Pro Glu Arg Ala Lys Thr Arg Lys  
                     610                      615                      620  
 Asn Asp Lys Thr His Arg Ile Asp Thr Thr Arg Gln Pro Leu Asp Pro  
 625                      630                      635                      640  
 Asp Val Ser Glu Asn Val Thr Lys Thr Cys Cys Leu Ser Val Ser Leu  
                     645                      650                      655  
 Thr Lys Trp Ala Lys Asn Gln Tyr Ala Pro Phe Ile Met Arg Pro Ala  
                     660                      665                      670  
 Val Lys Val Thr Ser Met Leu Ala Leu Ile Ala Val Ile Leu Thr Ser  
                     675                      680                      685  
 Val Trp Gly Ala Thr Lys Val Lys Asp Gly Leu Asp Leu Thr Asp Ile  
                     690                      695                      700  
 Val Pro Glu Asn Thr Asp Glu His Glu Phe Leu Ser Arg Gln Glu Lys  
 705                      710                      715                      720  
 Tyr Phe Gly Phe Tyr Asn Met Tyr Ala Val Thr Gln Gly Asn Phe Glu  
                     725                      730                      735  
 Tyr Pro Thr Asn Gln Lys Leu Leu Tyr Glu Tyr His Asp Gln Phe Val  
                     740                      745                      750  
 Arg Ile Pro Asn Ile Ile Lys Asn Asp Asn Gly Gly Leu Thr Lys Phe  
                     755                      760                      765

Trp Leu Ser Leu Phe Arg Asp Trp Leu Leu Asp Leu Gln Val Ala Phe  
 770 775 780  
 Asp Lys Glu Val Ala Ser Gly Cys Ile Thr Gln Glu Tyr Trp Cys Lys  
 785 790 795 800  
 Asn Ala Ser Asp Glu Gly Ile Leu Ala Tyr Lys Leu Met Val Gln Thr  
 805 810 815  
 Gly His Val Asp Asn Pro Ile Asp Lys Ser Leu Ile Thr Ala Gly His  
 820 825 830  
 Arg Leu Val Asp Lys Asp Gly Ile Ile Asn Pro Lys Ala Phe Tyr Asn  
 835 840 845  
 Tyr Leu Ser Ala Trp Ala Thr Asn Asp Ala Leu Ala Tyr Gly Ala Ser  
 850 855 860  
 Gln Gly Asn Leu Lys Pro Gln Pro Gln Arg Trp Ile His Ser Pro Glu  
 865 870 875 880  
 Asp Val His Leu Glu Ile Lys Lys Ser Ser Pro Leu Ile Tyr Thr Gln  
 885 890 895  
 Leu Pro Phe Tyr Leu Ser Gly Leu Ser Asp Thr Xaa Ser Ile Lys Thr  
 900 905 910  
 Leu Ile Arg Ser Val Arg Asp Leu Cys Leu Lys Tyr Glu Ala Lys Gly  
 915 920 925  
 Leu Pro Asn Phe Pro Ser Gly Ile Pro Phe Leu Phe Trp Glu Gln Tyr  
 930 935 940  
 Leu Tyr Leu Arg Thr Ser Leu Leu Leu Ala Leu Ala Cys Ala Leu Ala  
 945 950 955 960  
 Ala Val Phe Ile Ala Val Met Val Leu Leu Leu Asn Ala Trp Ala Ala  
 965 970 975  
 Val Leu Val Thr Leu Ala Leu Ala Thr Leu Val Leu Gln Leu Leu Gly  
 980 985 990  
 Val Met Ala Leu Leu Gly Val Lys Leu Ser Ala Met Pro Ala Val Leu  
 995 1000 1005  
 Leu Val Leu Ala Ile Gly Arg Gly Val His Phe Thr Val His Leu Cys  
 1010 1015 1020  
 Leu Gly Phe Val Thr Ser Ile Gly Cys Lys Arg Arg Arg Ala Ser Leu  
 1025 1030 1035 1040  
 Ala Leu Glu Ser Val Leu Ala Pro Val Val His Gly Ala Leu Ala Ala  
 1045 1050 1055  
 Ala Leu Ala Ala Ser Met Leu Ala Ala Ser Glu Cys Gly Phe Val Ala  
 1060 1065 1070  
 Arg Leu Phe Leu Arg Leu Leu Leu Asp Ile Val Phe Leu Gly Leu Ile

1075	1080	1085
Asp Gly Leu Leu Phe Phe Pro Ile Val Leu Ser Ile Leu Gly Pro Ala		
1090	1095	1100
Ala Glu Val Arg Pro Ile Glu His Pro Glu Arg Leu Ser Thr Pro Ser		
1105	1110	1115
Pro Lys Cys Ser Pro Ile His Pro Arg Lys Ser Ser Ser Ser Ser Gly		
	1125	1130
Gly Gly Asp Lys Ser Ser Arg Thr Ser Lys Ser Ala Pro Arg Pro Cys		
	1140	1145
Ala Pro Ser Leu Thr Thr Ile Thr Glu Glu Pro Ser Ser Trp His Ser		
	1155	1160
Ser Ala His Ser Val Gln Ser Ser Met Gln Ser Ile Val Val Gln Pro		
	1170	1175
Glu Val Val Val Glu Thr Thr Thr Tyr Asn Gly Ser Asp Ser Ala Ser		
	1185	1190
Gly Arg Ser Thr Pro Thr Lys Ser Ser His Gly Gly Ala Ile Thr Thr		
	1205	1210
Thr Lys Val Thr Ala Thr Ala Asn Ile Lys Val Glu Val Val Thr Pro		
	1220	1225
Ser Asp Arg Lys Ser Arg Arg Ser Tyr His Tyr Tyr Asp Arg Arg Arg		
	1235	1240
Asp Arg Asp Glu Asp Arg Asp Arg Asp Arg Glu Arg Asp Arg Asp Arg		
	1250	1255
Asp Arg Asp Arg Asp Arg Asp Arg Asp Arg Asp Arg Asp Arg Asp Arg		
	1265	1270
Glu Arg Ser Arg Glu Arg Asp Arg Arg Asp Arg Tyr Arg Asp Glu Arg		
	1285	1290
Asp His Arg Ala Ser Pro Arg Glu Lys Arg Gln Arg Phe Trp Thr		
	1300	1305

## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 4434 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGAAACAAGA GAGCGAGTGA GAGTAGGGAG AGCGTCTGTG TTGTGTGTTG AGTGTGCCCC	60
ACGCACACAG GCGCAAAACA GTGCACACAG ACGCCCGCTG GGCAAGAGAG AGTGAGAGAG	120
AGAAACAGCG GCGCGCGCTC GCCTAATGAA GTTGTGCGCC TGGCTGGCGT GCCGCATCCA	180
CGAGATACAG ATACATCTCT CATGGACCGC GACAGCCTCC CACGCGTTCC GGACACACAC	240
GGCGATGTGG TCGATGAGAA ATTATTCTCG GATCTTTACA TACGCACCAG CTGGGTGGAC	300
GCCCAAGTGG CGCTCGATCA GATAGATAAG GGCAAGCGC GTGGCAGCCG CACGGCGATC	360
TATCTGCGAT CAGTATTCCA GTCCACCTC GAAACCCTCG GCAGCTCCGT GCAAAAGCAC	420
GCGGGCAAGG TGCTATTCTG GGCTATCCTG GTGCTGAGCA CCTTCTGCGT CGGCCTGAAG	480
AGCGCCCGA TCCACTCCAA GGTGCACCAG CTGTGGATCC AGGAGGGCGG CCGGCTGGAG	540
GCGGAAGTGG CCTACACACA GAAGACGATC GGCGAGGACG AGTCGGCCAC GCATCAGCTG	600
CTCATTGAGA CGACCCACGA CCCGAACGCC TCCGTCTGTC ATCCGCAGGC GCTGCTTGCC	660
CACCTGGAGG TCCTGGTCAA GGCACCGCC GTCAAGGTGC ACCTCTACGA CACCGAATGG	720
GGGCTGCGCG ACATGTGCAA CATGCCGAGC ACGCCCTCCT TCGAGGGCAT CTACTACATC	780
GAGCAGATCC TGCGCCACCT CATTCCGTGC TCGATCATCA CGCCGCTGGA CTGTTTCTGG	840
GAGGGAAGCC AGCTGTTGGG TCCGGAATCA GCGGTCTTA TACCAGGCCT CAACCAACGA	900
CTCCTGTGGA CCACCCTGAA TCCCGCTCT GTGATGCAGT ATATGAAACA AAAGATGTCC	960
GAGGAAAAGA TCAGCTTCGA CTTGAGACC GTGGAGCAGT ACATGAAGCG TGCGGCCATT	1020
GGCAGTGGCT ACATGGAGAA GCCCTGCCTG AACCCTACTGA ATCCCAATTG CCGGACACG	1080
GCACCGAACA AGAACAGCAC CCAGCCGCGG GATGTGGGAG CCATCCTGTC CGGAGGCTGC	1140
TACGGTTATG CCGGAAGCA CATGCACTGG CCGGAGGAGC TGATTGTGGG CGGACGGAAG	1200
AGGAACCGCA GCGGACACTT GAGGAAGGCC CAGGCCCTGC AGTCGGTGGT GCAGCTGATG	1260
ACCGAGAAGG AAATGTACGA CCAGTGGCAG GACAATAA AGGTGCACCA TCTTGATGG	1320
ACGCAGGAGA AGGCAGCGGA GGTTTTGAAC GCCTGGCAGC GCAACTTTTC GCGGGAGGTG	1380
GAACAGCTGC TACGTAAACA GTCGAGAATT GCCACCAACT ACGATATCTA CGTGTTACAG	1440
TGGCTGTCAC TGGATGACAT CCTGGCCAAG TTCTCCCATC CCAGCGCCTT GTCCATTGTC	1500
ATCGGCGTGG CCGTCACCGT TTTGTATGCC TTTTGACGCG TCCTCCGCTG GAGGGACCCC	1560
GTCCGTGGCC AGAGCAGTGT GGGCGTGGCC GGAGTTCTGC TCATGTGCTT CAGTACCGCC	1620
GCCGGATTGG GATTGTCAGC CCTGCTCGGT ATCGTTTCA ATGCGCTGAC CGCTGCCTAT	1680
GCGGAGAGCA ATCGGCGGGA GCAGACCAAG CTGATTCTCA AGAACGCCAG CACCCAGGTG	1740

GTTCCGTTTT TGGCCCTTGG TCTGGGCGTC GATCACATCT TCATAGTGGG ACCGAGCATC 1800  
 CTGTTCAAGT CCTGCAGCAC CGCAGGATCC TTCTTTGCGG CCGCCTTTAT TCCGGTGCCG 1860  
 GCTTTGAAGG TATTCTGTCT GCAGGCTGCC ATCGTAATGT GCTCCAATTT GGCAGCGGCT 1920  
 CTATTGGTTT TTCGGGCCAT GATTTCGTTG GATCTACCGA GACGTACCGC CGGCAGGGCG 1980  
 GACATCTTCT GCTGCTGTTT TCCGGTGTGG AAGGAACAGC CGAAGGTGGC ACCTCCGGTG 2040  
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 AGGGTGCCGC TGCCCGCCCA GAATCCTCTG CTGGAACAGA GGCAGACAT CCCTGGGAGC 2160  
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 CTCATGCGCA GCTGGGTGAA GTTCTGACC GTTATGGGTT TCCTGGCGGC CCTCATATCC 2280  
 AGCTTGATG CCTCCACGCG CTTTCAAGAT GGCCTGGACA TTATTGATCT GGTGCCCAAG 2340  
 GACAGCAACG AGCACAAGTT CCTGGATGCT CAACTCCGC TCTTTGGCTT CTACAGCATG 2400  
 TATGCGGTTA CCCAGGGCAA CTTTGAATAT CCCACCCAGC AGCAGTTGCT CAGGGACTAC 2460  
 CATGATTCCT TTGTGCGGGT GCCACATGTG ATCAAGAATG ATAACGGTGG ACTGCCGGAC 2520  
 TTCTGGCTGC TGCTCTTCAG CGAGTGGCTG GGTAACTCTG AAAAGATATT CGACGAGGAA 2580  
 TACCGCGACG GACGGCTGAC CAAGGAGTGC TGGTTCCCAA ACGCCAGCAG CGATGCCATC 2640  
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 GTGCTACCA ATCGCTGGT CAACAGCGAT GGCATCATCA ACCAACGCGC CTTCTACAAC 2760  
 TATCTGTGG CATGGGCCAC CAACGACGTC TTGCGCTACG GAGCTTCTCA GGGCAAATTG 2820  
 TATCCGGAAC CGCGCCAGTA TTTTCACCAA CCCAACGAGT ACCATCTTAA GATACCCAAG 2880  
 AGTCTGCCAT TGGTCTACGC TCAGATGCCC TTTTACCTCC ACGGACTAAC AGATACCTCG 2940  
 CAGATCAAGA CCCTGATAGG TCATATTCGC GACCTGAGCG TCAAGTACGA GGGCTTCGGC 3000  
 CTGCCCAACT ATCCATCGGG CATTCCTTC ATCTTCTGGG AGCAGTACAT GACCCTGCGC 3060  
 TCCTCACTGG CCATGATCCT GGCCTGCGTG CTACTCGCCG CCCTGGTGCT GGTCTCCCTG 3120  
 CTCCTGCTCT CGGTTTGGGC CGCCGTTCTC GTGATCCTCA GCGTTCTGGC CTCGCTGGCC 3180  
 CAGATCTTTG GGGCCATGAC TCTGCTGGGC ATCAAACCTCT CGGCCATTCC GGCAGTCATA 3240  
 CTCATCCTCA GCGTGGGCAT GATGCTGTGC TTCAATGTGC TGATATCACT GGGCTTCATG 3300  
 ACATCCGTTG GCAACGACA GCGCCGCGTC CAGCTGAGCA TGCAGATGTC CCTGGGACCA 3360  
 CTTGTCCACG GCATGCTGAC CTCGGGAGTG GCCGTGTTCA TGCTCTCCAC GTCGCCCTTT 3420  
 GAGTTTGTGA TCCGGCACTT CTGCTGGCTT CTGCTGGTGG TCTTATGCGT TGGCGCCTGC 3480

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AACAGCCTTT TGGTGTTCCT CATCCTACTG AGCATGGTGG GACCGGAGGC GGAGCTGGTG      3540
CCGCTGGAGC ATCCAGACCG CATATCCACG CCCTCTCCGC TGCCCGTGCG CAGCAGCAAG      3600
AGATCGGGCA AATCCTATGT GGTGCAGGGA TCGCGATCCT CGCGAGGCAG CTGCCAGAAG      3660
TCGCATCACC ACCACCACAA AGACCTTAAT GATCCATCGC TGACGACGAT CACCGAGGAG      3720
CCGCAGTCGT GGAAGTCCAG CAACTCGTCC ATCCAGATGC CCAATGATTG GACCTACCAG      3780
CCGCGGGAAC AGCGACCGGC CTCCTACGGC GCGCGCCCCC CCGCCTATCA CAAGGCCGCC      3840
GCCCAGCAGC ACCACCAGCA TCAGGGCCCC CCCACAACGC CCGCGCCTCC CTTCCCCGACG      3900
GCCTATCCGC CGGAGCTGCA GAGCATCGTG GTGCAGCCGG AGGTGACGGT GGAGACGACG      3960
CACTCGGACA GCAACACCAC CAAGGTGACG GCCACGGCCA ACATCAAGGT GGAGCTGGCC      4020
ATGCCCGGCA GGGCGGTGCG CAGCTATAAC TTTACGAGTT AGCACTAGCA CTAGTTCCTG      4080
TAGCTATTAG GACGTATCTT TAGACTCTAG CCTAAGCCGT AACCCTATTT GTATCTGTAA      4140
AATCGATTTG TCCAGCGGGT CTGCTGAGGA TTTCGTTCTC ATGGATTCTC ATGGATTCTC      4200
ATGGATGCTT AAATGGCATG GTAATTGGCA AAATATCAAT TTTTGTGTCT CAAAAGATG      4260
CATTAGCTTA TGGTTTCAAG ATACATTTT AAAGAGTCCG CCAGATATTT ATATAAAAAA      4320
AATCCAAAT CGACGTATCC ATGAAAATTG AAAAGCTAAG CAGACCCGTA TGTATGTATA      4380
TGTGTATGCA TGTTAGTTAA TTTCCCGAAG TCCGGTATTT ATAGCAGCTG CCTT      4434

```

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1285 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Met Asp Arg Asp Ser Leu Pro Arg Val Pro Asp Thr His Gly Asp Val
1           5           10           15
Val Asp Glu Lys Leu Phe Ser Asp Leu Tyr Ile Arg Thr Ser Trp Val
20           25           30
Asp Ala Gln Val Ala Leu Asp Gln Ile Asp Lys Gly Lys Ala Arg Gly
35           40           45
Ser Arg Thr Ala Ile Tyr Leu Arg Ser Val Phe Gln Ser His Leu Glu
50           55           60

```

Thr Leu Gly Ser Ser Val Gln Lys His Ala Gly Lys Val Leu Phe Val  
 65 70 75 80  
 Ala Ile Leu Val Leu Ser Thr Phe Cys Val Gly Leu Lys Ser Ala Gln  
 85 90 95  
 Ile His Ser Lys Val His Gln Leu Trp Ile Gln Glu Gly Gly Arg Leu  
 100 105 110  
 Glu Ala Glu Leu Ala Tyr Thr Gln Lys Thr Ile Gly Glu Asp Glu Ser  
 115 120 125  
 Ala Thr His Gln Leu Leu Ile Gln Thr Thr His Asp Pro Asn Ala Ser  
 130 135 140  
 Val Leu His Pro Gln Ala Leu Leu Ala His Leu Glu Val Leu Val Lys  
 145 150 155 160  
 Ala Thr Ala Val Lys Val His Leu Tyr Asp Thr Glu Trp Gly Leu Arg  
 165 170 175  
 Asp Met Cys Asn Met Pro Ser Thr Pro Ser Phe Glu Gly Ile Tyr Tyr  
 180 185 190  
 Ile Glu Gln Ile Leu Arg His Leu Ile Pro Cys Ser Ile Ile Thr Pro  
 195 200 205  
 Leu Asp Cys Phe Trp Glu Gly Ser Gln Leu Leu Gly Pro Glu Ser Ala  
 210 215 220  
 Val Val Ile Pro Gly Leu Asn Gln Arg Leu Leu Trp Thr Thr Leu Asn  
 225 230 235 240  
 Pro Ala Ser Val Met Gln Tyr Met Lys Gln Lys Met Ser Glu Glu Lys  
 245 250 255  
 Ile Ser Phe Asp Phe Glu Thr Val Glu Gln Tyr Met Lys Arg Ala Ala  
 260 265 270  
 Ile Gly Ser Gly Tyr Met Glu Lys Pro Cys Leu Asn Pro Leu Asn Pro  
 275 280 285  
 Asn Cys Pro Asp Thr Ala Pro Asn Lys Asn Ser Thr Gln Pro Pro Asp  
 290 295 300  
 Val Gly Ala Ile Leu Ser Gly Gly Cys Tyr Gly Tyr Ala Ala Lys His  
 305 310 315 320  
 Met His Trp Pro Glu Glu Leu Ile Val Gly Gly Arg Lys Arg Asn Arg  
 325 330 335  
 Ser Gly His Leu Arg Lys Ala Gln Ala Leu Gln Ser Val Val Gln Leu  
 340 345 350  
 Met Thr Glu Lys Glu Met Tyr Asp Gln Trp Gln Asp Asn Tyr Lys Val  
 355 360 365  
 His His Leu Gly Trp Thr Gln Glu Lys Ala Ala Glu Val Leu Asn Ala



370	375	380
Trp Gln Arg Asn Phe Ser Arg Glu Val Glu Gln Leu Leu Arg Lys Gln 385 390 395 400		
Ser Arg Ile Ala Thr Asn Tyr Asp Ile Tyr Val Phe Ser Ser Ala Ala 405 410 415		
Leu Asp Asp Ile Leu Ala Lys Phe Ser His Pro Ser Ala Leu Ser Ile 420 425 430		
Val Ile Gly Val Ala Val Thr Val Leu Tyr Ala Phe Cys Thr Leu Leu 435 440 445		
Arg Trp Arg Asp Pro Val Arg Gly Gln Ser Ser Val Gly Val Ala Gly 450 455 460		
Val Leu Leu Met Cys Phe Ser Thr Ala Ala Gly Leu Gly Leu Ser Ala 465 470 475 480		
Leu Leu Gly Ile Val Phe Asn Ala Leu Thr Ala Ala Tyr Ala Glu Ser 485 490 495		
Asn Arg Arg Glu Gln Thr Lys Leu Ile Leu Lys Asn Ala Ser Thr Gln 500 505 510		
Val Val Pro Phe Leu Ala Leu Gly Leu Gly Val Asp His Ile Phe Ile 515 520 525		
Val Gly Pro Ser Ile Leu Phe Ser Ala Cys Ser Thr Ala Gly Ser Phe 530 535 540		
Phe Ala Ala Ala Phe Ile Pro Val Pro Ala Leu Lys Val Phe Cys Leu 545 550 555 560		
Gln Ala Ala Ile Val Met Cys Ser Asn Leu Ala Ala Ala Leu Leu Val 565 570 575		
Phe Pro Ala Met Ile Ser Leu Asp Leu Arg Arg Arg Thr Ala Gly Arg 580 585 590		
Ala Asp Ile Phe Cys Cys Cys Phe Pro Val Trp Lys Glu Gln Pro Lys 595 600 605		
Val Ala Pro Pro Val Leu Pro Leu Asn Asn Asn Asn Gly Arg Gly Ala 610 615 620		
Arg His Pro Lys Ser Cys Asn Asn Asn Arg Val Pro Leu Pro Ala Gln 625 630 635 640		
Asn Pro Leu Leu Glu Gln Arg Ala Asp Ile Pro Gly Ser Ser His Ser 645 650 655		
Leu Ala Ser Phe Ser Leu Ala Thr Phe Ala Phe Gln His Tyr Thr Pro 660 665 670		
Phe Leu Met Arg Ser Trp Val Lys Phe Leu Thr Val Met Gly Phe Leu 675 680 685		

WO 96/11260

Ala Ala Leu Ile Ser Ser Leu Tyr Ala Ser Thr Arg Leu Gln Asp Gly  
 690 695 700  
 Leu Asp Ile Ile Asp Leu Val Pro Lys Asp Ser Asn Glu His Lys Phe  
 705 710 715 720  
 Leu Asp Ala Gln Thr Arg Leu Phe Gly Phe Tyr Ser Met Tyr Ala Val  
 725 730 735  
 Thr Gln Gly Asn Phe Glu Tyr Pro Thr Gln Gln Gln Leu Leu Arg Asp  
 740 745 750  
 Tyr His Asp Ser Phe Arg Val Pro His Val Ile Lys Asn Asp Asn Gly  
 755 760 765  
 Gly Leu Pro Asp Phe Trp Leu Leu Leu Phe Ser Glu Trp Leu Gly Asn  
 770 775 780  
 Leu Gln Lys Ile Phe Asp Glu Glu Tyr Arg Asp Gly Arg Leu Thr Lys  
 785 790 795 800  
 Glu Cys Trp Phe Pro Asn Ala Ser Ser Asp Ala Ile Leu Ala Tyr Lys  
 805 810 815  
 Leu Ile Val Gln Thr Gly His Val Asp Asn Pro Val Asp Lys Glu Leu  
 820 825 830  
 Val Leu Thr Asn Arg Leu Val Asn Ser Asp Gly Ile Ile Asn Gln Arg  
 835 840 845  
 Ala Phe Tyr Asn Tyr Leu Ser Ala Trp Ala Thr Asn Asp Val Phe Ala  
 850 855 860  
 Tyr Gly Ala Ser Gln Gly Lys Leu Tyr Pro Glu Pro Arg Gln Tyr Phe  
 865 870 875 880  
 His Gln Pro Asn Glu Tyr Asp Leu Lys Ile Pro Lys Ser Leu Pro Leu  
 885 890 895  
 Val Tyr Ala Gln Met Pro Phe Tyr Leu His Gly Leu Thr Asp Thr Ser  
 900 905 910  
 Gln Ile Lys Thr Leu Ile Gly His Ile Arg Asp Leu Ser Val Lys Tyr  
 915 920 925  
 Glu Gly Phe Gly Leu Pro Asn Tyr Pro Ser Gly Ile Pro Phe Ile Phe  
 930 935 940  
 Trp Glu Gln Tyr Met Thr Leu Arg Ser Ser Leu Ala Met Ile Leu Ala  
 945 950 955 960  
 Cys Val Leu Leu Ala Ala Leu Val Leu Val Ser Leu Leu Leu Ser  
 965 970 975  
 Val Trp Ala Ala Val Leu Val Ile Leu Ser Val Leu Ala Ser Leu Ala  
 980 985 990  
 Gln Ile Phe Gly Ala Met Thr Leu Leu Gly Ile Lys Leu Ser Ala Ile

995	1000	1005
Pro Ala Val Ile Leu Ile Leu Ser Val Gly Met Met Leu Cys Phe Asn 1010	1015	1020
Val Leu Ile Ser Leu Gly Phe Met Thr Ser Val Gly Asn Arg Gln Arg 1025	1030	1035 1040
Arg Val Gln Leu Ser Met Gln Met Ser Leu Gly Pro Leu Val His Gly 1045	1050	1055
Met Leu Thr Ser Gly Val Ala Val Phe Met Leu Ser Thr Ser Pro Phe 1060	1065	1070
Glu Phe Val Ile Arg His Phe Cys Trp Leu Leu Leu Val Val Leu Cys 1075	1080	1085
Val Gly Ala Cys Asn Ser Leu Leu Val Phe Pro Ile Leu Leu Ser Met 1090	1095	1100
Val Gly Pro Glu Ala Glu Leu Val Pro Leu Glu His Pro Asp Arg Ile 1105	1110	1115 1120
Ser Thr Pro Ser Pro Leu Pro Val Arg Ser Ser Lys Arg Ser Gly Lys 1125	1130	1135
Ser Tyr Val Val Gln Gly Ser Arg Ser Ser Arg Gly Ser Cys Gln Lys 1140	1145	1150
Ser His His His His His Lys Asp Leu Asn Asp Pro Ser Leu Thr Thr 1155	1160	1165
Ile Thr Glu Glu Pro Gln Ser Trp Lys Ser Ser Asn Ser Ser Ile Gln 1170	1175	1180
Met Pro Asn Asp Trp Thr Tyr Gln Pro Arg Glu Gln Arg Pro Ala Ser 1185	1190	1195 1200
Tyr Ala Ala Pro Pro Pro Ala Tyr His Lys Ala Ala Ala Gln Gln His 1205	1210	1215
His Gln His Gln Gly Pro Pro Thr Thr Pro Pro Pro Pro Phe Pro Thr 1220	1225	1230
Ala Tyr Pro Pro Glu Leu Gln Ser Ile Val Val Gln Pro Glu Val Thr 1235	1240	1245
Val Glu Thr Thr His Ser Asp Ser Asn Thr Thr Lys Val Thr Ala Thr 1250	1255	1260
Ala Asn Ile Lys Val Glu Leu Ala Met Pro Gly Arg Ala Val Arg Ser 1265	1270	1275 1280
Tyr Asn Phe Thr Ser 1285		

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 345 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```

AAGGTCCATC AGCTTTGGAT ACAGGAAGGT GGTTCGCTCG AGCATGAGCT AGCCTACACG      60
CAGAAATCGC TCGGCGAGAT GGACTCCTCC ACGCACCAGC TGCTAATCCA AACNCCCAAA      120
GATATGGACG CCTCGATACT GCACCCGAAC GCGCTACTGA CGCACCTGGA CGTGGTGAAG      180
AAAGCGATCT CGGTGACGGT GCACATGTAC GACATCACGT GGAGNCTCAA GGACATGTGC      240
TACTCGCCCA GCATACCGAG NTTGATACG CACTTTATCG AGCAGATCTT CGAGAACATC      300
ATACCGTGCG CGATCATCAC GCCGCTGGAT TGCTTTGGG AGGGA                        345
  
```

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 115 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```

Lys Val His Gln Leu Trp Ile Gln Glu Gly Gly Ser Leu Glu His Glu
1           5           10           15
Leu Ala Tyr Thr Gln Lys Ser Leu Gly Glu Met Asp Ser Ser Thr His
20          25          30
Gln Leu Leu Ile Gln Thr Pro Lys Asp Met Asp Ala Ser Ile Leu His
35          40          45
Pro Asn Ala Leu Leu Thr His Leu Asp Val Val Lys Lys Ala Ile Ser
50          55          60
Val Thr Val His Met Tyr Asp Ile Thr Trp Xaa Leu Lys Asp Met Cys
65          70          75          80
Tyr Ser Pro Ser Ile Pro Xaa Phe Asp Thr His Phe Ile Glu Gln Ile
85          90          95
  
```

Phe Glu Asn Ile Ile Pro Cys Ala Ile Ile Thr Pro Leu Asp Cys Phe  
 100 105 110

Trp Glu Gly  
 115

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 5187 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGGTCTGTCA CCCGGAGCCG GAGTCCCCGG CGGCCAGCAG CGTCCTCGCG AGCCGAGCGC	60
CCAGGCGCGC CCGGAGCCCG CGGCGGCGGC GGCAACATGG CCTCGGCTGG TAACGCCGCC	120
GGGGCCCTGG GCAGGCAGGC CGGCGGCGGG AGGCGCAGAC GGACCGGGGG ACCGCACCGC	180
GCCGCGCCGG ACCGGGACTA TCTGCACCGG CCCAGCTACT GCGACGCCGC CTTGCTCTG	240
GAGCAGATTT CCAAGGGGAA GGCTACTGGC CGGAAAGCGC CGCTGTGGCT GAGAGCGAAG	300
TTTCAGAGAC TCTTATTTAA ACTGGGTTGT TACATTCAA AGAACTGCGG CAAGTTTTTG	360
GTTGTGGGTC TCCTCATATT TGGGGCCTTC GCTGTGGGAT TAAAGGCAGC TAATCTCGAG	420
ACCAACGTGG AGGAGCTGTG GGTGGAAGTT GGTGGACGAG TGAGTCGAGA ATTAAATTAT	480
ACCCGTCAGA AGATAGGAGA AGAGGCTATG TTTAATCCTC AACTCATGAT ACAGACTCCA	540
AAAGAAGAAG GCGCTAATGT TCTGACCACA GAGGCTCTCC TGCAACACCT GGAATCAGCA	600
CTCCAGGCCA GTCGTGTGCA CGTCTACATG TATAACAGGC AATGGAAGTT GGAACATTG	660
TGCTACAAAT CAGGGGAACT TATCAGGAG ACAGGTTACA TGGATCAGAT AATAGAATAC	720
CTTTACCCTT GCTTAATCAT TACACCTTG GACTGCTTCT GGAAGGGGC AAAGCTACAG	780
TCCGGGACAG CATACTCCT AGGTAAGCCT CCTTTACGGT GGACAACTT TGACCCCTTG	840
GAATTCCTAG AAGAGTTAAA GAAAATAAAC TACCAAGTGG ACAGCTGGGA GGAAATGCTG	900
AATAAGCCG AAGTTGGCCA TGGGTACATG GACCGGCCTT GCCTCAACCC AGCCGACCCA	960
GATTGCCCTG CCACAGCCCC TAACAAAAAT TCAACCAAAC CTCTTGATGT GGCCCTTGTT	1020
TTGAATGGTG GATGTCAAGG TTTATCCAGG AAGTATATGC ATTGGCAGGA GGAGTTGATT	1080
GTGGGTGGTA CCGTCAAGAA TGCCACTGGA AAAGTTGTCA GCGCTCACGC CCTGCAAACC	1140

ATGTTCCAGT TAATGACTCC CAAGCAAATG TATGAACACT TCAGGGGCTA CGACTATGTC	1200
TCTCACATCA ACTGGAATGA AGACAGGGCA GCCGCCATCC TGGAGGCCTG GCAGAGGACT	1260
TACGTGGAGG TGGTTCATCA AAGTGTGCCC CCAAACCTCCA CTCAAAAGGT GCTTCCCTTC	1320
ACAACCACGA CCCTGGACGA CATCCTAAAA TCCTTCTCTG ATGTCAGTGT CATCCGAGTG	1380
GCCAGCGGCT ACCTACTGAT GCTTGCCTAT GCCTGTTTAA CCATGCTGCG CTGGGACTGC	1440
TCCAAGTCCC AGGGTGCCGT GGGGCTGGCT GGCCTCCTGT TGGTTGCGCT GTCAGTGGCT	1500
GCAGGATTGG GCCTCTGCTC CTTGATTGGC ATTTCTTTTA ATGCTGCGAC AACTCAGGTT	1560
TTGCCGTTTC TTGCTCTTGG TGTTGGTGTG GATGATGTCT TCCTCCTGGC CCATGCATTC	1620
AGTGAAACAG GACAGAATAA GAGGATTCCA TTTGAGGACA GGACTGGGGA GTGCCTCAAG	1680
CGCACCGGAG CCAGCGTGGC CCTCACCTCC ATCAGCAATG TCACCGCCTT CTTCATGGCC	1740
GCATTGATCC CTATCCCTGC CCTGCGAGCG TTCTCCCTCC AGGCTGCTGT GGTGGTGGTA	1800
TTCAATTTTG CTATGGTTCT GCTCATTTTT CCTGCAATTC TCAGCATGGA TTTATACAGA	1860
CGTGAGGACA GAAGATTGGA TATTTTCTGC TGTTTCACAA GCCCCTGTGT CAGCAGGGTG	1920
ATTCAAGTTG AGCCACAGGC CTACACAGAG CCTCACAGTA ACACCCGGTA CAGCCCCCA	1980
CCCCATACA CCAGCCACAG CTTGCCCCAC GAAACCCATA TCACTATGCA GTCCACCGTT	2040
CAGCTCCGCA CAGAGTATGA CCCTCACAGC CACGTGTACT ACACCACCGC CGAGCCACGC	2100
TCTGAGATCT CTGTACAGCC TGTTACCGTC ACCCAGGACA ACCTCAGCTG TCAGAGTCCC	2160
GAGAGCACCA GCTCTACCAG GGACCTGCTC TCCCAGTTCT CAGACTCCAG CCTCCACTGC	2220
CTCGAGCCCC CCTGCACCAA GTGGACACTC TCTTCGTTTG CAGAGAAGCA CTATGCTCCT	2280
TTCCTCCTGA AACCCAAAGC CAAGGTTGTG GTAATCCTTC TTTTCCTGGG CTGCTGGGG	2340
GTCAGCCTTT ATGGGACCAC CCGAGTGAGA GACGGGCTGG ACCTCACGGA CATTGTTCCC	2400
CGGGAAACCA GAGAATATGA CTTCATAGCT GCCCAGTTCA AGTACTTCTC TTTCTACAAC	2460
ATGTATATAG TCACCCAGAA AGCAGACTAC CCGAATATCC AGCACCTACT TTACGACCTT	2520
CATAAGAGTT TCAGCAATGT GAAGTATGTC ATGCTGGAGG AGAACAAGCA ACTTCCCCAA	2580
ATGTGGCTGC ACTACTTTAG AGACTGGCTT CAAGGACTTC AGGATGCATT TGACAGTGAC	2640
TGGGAAACTG GGAGGATCAT GCCAAACAAT TATAAAAATG GATCAGATGA CGGGGTCCTC	2700
GCTTACAAAC TCCTGGTGCA GACTGGCAGC CGAGACAAGC CCATCGACAT TAGTCAGTTG	2760
ACTAAACAGC GTCTGGTAGA CGCAGATGGC ATCATTAATC CGAGCGCTTT CTACATCTAC	2820
CTGACCGCTT GGGTCAGCAA CGACCCTGTA GCTTACGCTG CCTCCCAGGC CAACATCCGG	2880

CCTCACCGGC CGGAGTGGGT CCATGACAAA GCCGACTACA TGCCAGAGAC CAGGCTGAGA	2940
ATCCCAGCAG CAGAGCCCAT CGAGTACGCT CAGTTCCCTT TCTACCTCAA CGGCCTACGA	3000
GACACCTCAG ACTTTGTGGA AGCCATAGAA AAAGTGAGAG TCATCTGTAA CAACTATACG	3060
AGCCTGGGAC TGTCCAGCTA CCCC AATGGC TACCCCTTCC TGTTCTGGGA GCAATACATC	3120
AGCCTGCGCC ACTGGCTGCT GCTATCCATC AGCGTGGTGC TGGCCTGCAC GTTCTAGTG	3180
TGCGCAGTCT TCCTCCTGAA CCCCTGGACG GCGGGATCA TTGTCATGGT CCTGGCTCTG	3240
ATGACCGTTG AGCTCTTTGG CATGATGGGC CTCATTGGGA TCAAGCTGAG TGCTGTGCCT	3300
GTGGTCATCC TGATTGCATC TGTTGGCATC GGAGTGGAGT TCACCGTCCA CGTGGCTTTG	3360
GCCTTTCTGA CAGCCATTGG GGACAAGAAC CACAGGGCTA TGCTCGCTCT GGAACACATG	3420
TTTGCTCCCG TTCTGGACGG TGCTGTGTCC ACTCTGCTGG GTGTACTGAT GCTTGCAGGG	3480
TCCGAATTG ATTTCAATTG CAGATACTTC TTTGCCGTCC TGGCCATTCT CACCGTCTTG	3540
GGGGTTCTCA ATGGACTGGT TCTGCTGCCT GTCCTCTTAT CCTTCTTTGG ACCGTGTCTT	3600
GAGGTGTCTC CAGCCAATGG CCTAAACCGA CTGCCCACTC CTTGCGCTGA GCCGCCTCCA	3660
AGTGTGCTCC GGTTTGCCGT GCCTCCTGGT CACACGAACA ATGGGTCTGA TTCCTCCGAC	3720
TCGGAGTACA GCTCTCAGAC CACGGTGTCT GGCATCAGTG AGGAGCTCAG GCAATACGAA	3780
GCACAGCAGG GTGCCGGAGG CCCTGCCCAC CAAGTGATTG TGGAAGCCAC AGAAAACCTT	3840
GTCTTTGCCC GGTCCACTGT GGTCCATCCG GACTCCAGAC ATCAGCCTCC CTTGACCCCT	3900
CGGCAACAGC CCCACCTGGA CTCTGGCTCC TTGTCCCTG GACGGCAAGG CCAGCAGCCT	3960
CGAAGGGATC CCCCTAGAGA AGGCTTGCGG CCACCCCCCT ACAGACCGCG CAGAGACGCT	4020
TTTGAAATTT CTAAGAAGG GCATTCTGGC CTAAGAATA GGGACCGCTC AGGGCCCCGT	4080
GGGGCCCGTT CTCACAACCC TCGGAACCCA ACGTCCACCG CCATGGGCAG CTCTGTGCCC	4140
AGCTACTGCC AGCCCATCAC CACTGTGACG GCTTCTGCTT CGGTGACTGT TGCTGTGCAT	4200
CCCCCGCCTG GACCTGGGCG CAACCCCGA GGGGGGCCCT GTCCAGGCTA TGAGAGCTAC	4260
CCTGAGACTG ATCACGGGGT ATTTGAGGAT CCTCATGTGC CTTTTCATGT CAGGTGTGAG	4320
AGGAGGGACT CAAAGGTGGA GGTATAGAG CTACAGGACG TGAATGTGA GGAGAGGCCG	4380
TGGGGGAGCA GCTCCAATG AGGGTAATTA AAATCTGAAG CAAAGAGGCC AAAGATTGGA	4440
AAGCCCCGCC CCCACCTCTT TCCAGAACTG CTTGAAGAGA ACTGCTTGA ATTATGGGAA	4500
GGCAGTTCAT TGTTACTGTA ACTGATTGTA TTATTKKGTG AAATATTTCT ATAAATATTT	4560
AARAGGTGTA CACATGTAAT ATACATGGAA ATGCTGTACA GTCTATTTCC TGGGGCCTCT	4620

CCACTCCTGC CCCAGAGTGG GGAGACCACA GGGGCCCTTT CCCCTGTGTA CATTGGTCTC 4680  
 TGTGCCACAA CCAAGCTTAA CTTAGTTTTA AAAAAATCT CCCAGCATAT GTCGCTGCTG 4740  
 CTTAAATATT GTATAATTTA CTTGTATAAT TCTATGCAAA TATTGCTTAT GTAATAGGAT 4800  
 TATTTGTAAA GGTTCCTGTT TAAAATATTT TAAATTTGCA TATCACAACC CTGTGGTAGG 4860  
 ATGAATTGTT ACTGTTAACT TTTGAACACG CTATGCGTGG TAATTGTTTA ACGAGCAGAC 4920  
 ATGAAGAAAA CAGGTTAATC CCAGTGGCTT CTCTAGGGGT AGTTGTATAT GGTTCGCATG 4980  
 GGTGGATGTG TGTGTGCATG TGA CTTTCCA ATGTACTGTA TTGTGGTTTG TTGTTGTTGT 5040  
 TGCTGTTGTT GTTCATTTTG GTGTTTTTGG TTGCTTTGTA TGATCTTAGC TCTGGCCTAG 5100  
 GTGGGCTGGG AAGGTCCAGG TCTTTTTCTG TCGTGATGCT GGTGAAAGG TGACCCCAAT 5160  
 CATCTGTCCT ATTCTCTGGG ACTATTC 5187

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1434 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ala Ser Ala Gly Asn Ala Ala Gly Ala Leu Gly Arg Gln Ala Gly  
 1 5 10 15  
 Gly Gly Arg Arg Arg Arg Thr Gly Gly Pro His Arg Ala Ala Pro Asp  
 20 25 30  
 Arg Asp Tyr Leu His Arg Pro Ser Tyr Cys Asp Ala Ala Phe Ala Leu  
 35 40 45  
 Glu Gln Ile Ser Lys Gly Lys Ala Thr Gly Arg Lys Ala Pro Leu Trp  
 50 55 60  
 Leu Arg Ala Lys Phe Gln Arg Leu Leu Phe Lys Leu Gly Cys Tyr Ile  
 65 70 75 80  
 Gln Lys Asn Cys Gly Lys Phe Leu Val Val Gly Leu Leu Ile Phe Gly  
 85 90 95  
 Ala Phe Ala Val Gly Leu Lys Ala Ala Asn Leu Glu Thr Asn Val Glu  
 100 105 110  
 Glu Leu Trp Val Glu Val Gly Gly Arg Val Ser Arg Glu Leu Asn Tyr  
 115 120 125



Thr Arg Gln Lys Ile Gly Glu Glu Ala Met Phe Asn Pro Gln Leu Met  
 130 135 140  
 Ile Gln Thr Pro Lys Glu Glu Gly Ala Asn Val Leu Thr Thr Glu Ala  
 145 150 155 160  
 Leu Leu Gln His Leu Asp Ser Ala Leu Gln Ala Ser Arg Val His Val  
 165 170 175  
 Tyr Met Tyr Asn Arg Gln Trp Lys Leu Glu His Leu Cys Tyr Lys Ser  
 180 185 190  
 Gly Glu Leu Ile Thr Glu Thr Gly Tyr Met Asp Gln Ile Ile Glu Tyr  
 195 200 205  
 Leu Tyr Pro Cys Leu Ile Ile Thr Pro Leu Asp Cys Phe Trp Glu Gly  
 210 215 220  
 Ala Lys Leu Gln Ser Gly Thr Ala Tyr Leu Leu Gly Lys Pro Pro Leu  
 225 230 235 240  
 Arg Trp Thr Asn Phe Asp Pro Leu Glu Phe Leu Glu Glu Leu Lys Lys  
 245 250 255  
 Ile Asn Tyr Gln Val Asp Ser Trp Glu Glu Met Leu Asn Lys Ala Glu  
 260 265 270  
 Val Gly His Gly Tyr Met Asp Arg Pro Cys Leu Asn Pro Ala Asp Pro  
 275 280 285  
 Asp Cys Pro Ala Thr Ala Pro Asn Lys Asn Ser Thr Lys Pro Leu Asp  
 290 295 300  
 Val Ala Leu Val Leu Asn Gly Gly Cys Gln Gly Leu Ser Arg Lys Tyr  
 305 310 315 320  
 Met His Trp Gln Glu Glu Leu Ile Val Gly Gly Thr Val Lys Asn Ala  
 325 330 335  
 Thr Gly Lys Leu Val Ser Ala His Ala Leu Gln Thr Met Phe Gln Leu  
 340 345 350  
 Met Thr Pro Lys Gln Met Tyr Glu His Phe Arg Gly Tyr Asp Tyr Val  
 355 360 365  
 Ser His Ile Asn Trp Asn Glu Asp Arg Ala Ala Ala Ile Leu Glu Ala  
 370 375 380  
 Trp Gln Arg Thr Tyr Val Glu Val Val His Gln Ser Val Ala Pro Asn  
 385 390 395 400  
 Ser Thr Gln Lys Val Leu Pro Phe Thr Thr Thr Thr Leu Asp Asp Ile  
 405 410 415  
 Leu Lys Ser Phe Ser Asp Val Ser Val Ile Arg Val Ala Ser Gly Tyr  
 420 425 430  
 Leu Leu Met Leu Ala Tyr Ala Cys Leu Thr Met Leu Arg Trp Asp Cys

435	440	445
Ser Lys Ser Gln Gly Ala Val Gly Leu Ala Gly Val Leu Leu Val Ala 450	455	460
Leu Ser Val Ala Ala Gly Leu Gly Leu Cys Ser Leu Ile Gly Ile Ser 465	470	475 480
Phe Asn Ala Ala Thr Thr Gln Val Leu Pro Phe Leu Ala Leu Gly Val 485	490	495
Gly Val Asp Asp Val Phe Leu Leu Ala His Ala Phe Ser Glu Thr Gly 500	505	510
Gln Asn Lys Arg Ile Pro Phe Glu Asp Arg Thr Gly Glu Cys Leu Lys 515	520	525
Arg Thr Gly Ala Ser Val Ala Leu Thr Ser Ile Ser Asn Val Thr Ala 530	535	540
Phe Phe Met Ala Ala Leu Ile Pro Ile Pro Ala Leu Arg Ala Phe Ser 545	550	555 560
Leu Gln Ala Ala Val Val Val Val Phe Asn Phe Ala Met Val Leu Leu 565	570	575
Ile Phe Pro Ala Ile Leu Ser Met Asp Leu Tyr Arg Arg Glu Asp Arg 580	585	590
Arg Leu Asp Ile Phe Cys Cys Phe Thr Ser Pro Cys Val Ser Arg Val 595	600	605
Ile Gln Val Glu Pro Gln Ala Tyr Thr Glu Pro His Ser Asn Thr Arg 610	615	620
Tyr Ser Pro Pro Pro Pro Tyr Thr Ser His Ser Phe Ala His Glu Thr 625	630	635 640
His Ile Thr Met Gln Ser Thr Val Gln Leu Arg Thr Glu Tyr Asp Pro 645	650	655
His Thr His Val Tyr Tyr Thr Thr Ala Glu Pro Arg Ser Glu Ile Ser 660	665	670
Val Gln Pro Val Thr Val Thr Gln Asp Asn Leu Ser Cys Gln Ser Pro 675	680	685
Glu Ser Thr Ser Ser Thr Arg Asp Leu Leu Ser Gln Phe Ser Asp Ser 690	695	700
Ser Leu His Cys Leu Glu Pro Pro Cys Thr Lys Trp Thr Leu Ser Ser 705	710	715 720
Phe Ala Glu Lys His Tyr Ala Pro Phe Leu Leu Lys Pro Lys Ala Lys 725	730	735
Val Val Val Ile Leu Leu Phe Leu Gly Leu Leu Gly Val Ser Leu Tyr 740	745	750

Gly Thr Thr Arg Val Arg Asp Gly Leu Asp Leu Thr Asp Ile Val Pro  
 755 760 765  
 Arg Glu Thr Arg Glu Tyr Asp Phe Ile Ala Ala Gln Phe Lys Tyr Phe  
 770 775 780  
 Ser Phe Tyr Asn Met Tyr Ile Val Thr Gln Lys Ala Asp Tyr Pro Asn  
 785 790 795 800  
 Ile Gln His Leu Leu Tyr Asp Leu His Lys Ser Phe Ser Asn Val Lys  
 805 810 815  
 Tyr Val Met Leu Glu Glu Asn Lys Gln Leu Pro Gln Met Trp Leu His  
 820 825 830  
 Tyr Phe Arg Asp Trp Leu Gln Gly Leu Gln Asp Ala Phe Asp Ser Asp  
 835 840 845  
 Trp Glu Thr Gly Arg Ile Met Pro Asn Asn Tyr Lys Asn Gly Ser Asp  
 850 855 860  
 Asp Gly Val Leu Ala Tyr Lys Leu Leu Val Gln Thr Gly Ser Arg Asp  
 865 870 875 880  
 Lys Pro Ile Asp Ile Ser Gln Leu Thr Lys Gln Arg Leu Val Asp Ala  
 885 890 895  
 Asp Gly Ile Ile Asn Pro Ser Ala Phe Tyr Ile Tyr Leu Thr Ala Trp  
 900 905 910  
 Val Ser Asn Asp Pro Val Ala Tyr Ala Ala Ser Gln Ala Asn Ile Arg  
 915 920 925  
 Pro His Arg Pro Glu Trp Val His Asp Lys Ala Asp Tyr Met Pro Glu  
 930 935 940  
 Thr Arg Leu Arg Ile Pro Ala Ala Glu Pro Ile Glu Tyr Ala Gln Phe  
 945 950 955 960  
 Pro Phe Tyr Leu Asn Gly Leu Arg Asp Thr Ser Asp Phe Val Glu Ala  
 965 970 975  
 Ile Glu Lys Val Arg Val Ile Cys Asn Asn Tyr Thr Ser Leu Gly Leu  
 980 985 990  
 Ser Ser Tyr Pro Asn Gly Tyr Pro Phe Leu Phe Trp Glu Gln Tyr Ile  
 995 1000 1005  
 Ser Leu Arg His Trp Leu Leu Leu Ser Ile Ser Val Val Leu Ala Cys  
 1010 1015 1020  
 Thr Phe Leu Val Cys Ala Val Phe Leu Leu Asn Pro Trp Thr Ala Gly  
 1025 1030 1035 1040  
 Ile Ile Val Met Val Leu Ala Leu Met Thr Val Glu Leu Phe Gly Met  
 1045 1050 1055  
 Met Gly Leu Ile Gly Ile Lys Leu Ser Ala Val Pro Val Val Ile Leu

1060	1065	1070
Ile Ala Ser Val Gly	Ile Gly Val Glu Phe Thr Val His Val Ala Leu	
1075	1080	1085
Ala Phe Leu Thr Ala Ile Gly Asp Lys Asn His Arg Ala Met Leu Ala		
1090	1095	1100
Leu Glu His Met Phe Ala Pro Val Leu Asp Gly Ala Val Ser Thr Leu		
1105	1110	1115 1120
Leu Gly Val Leu Met Leu Ala Gly Ser Glu Phe Asp Phe Ile Val Arg		
1125	1130	1135
Tyr Phe Phe Ala Val Leu Ala Ile Leu Thr Val Leu Gly Val Leu Asn		
1140	1145	1150
Gly Leu Val Leu Leu Pro Val Leu Leu Ser Phe Phe Gly Pro Cys Pro		
1155	1160	1165
Glu Val Ser Pro Ala Asn Gly Leu Asn Arg Leu Pro Thr Pro Ser Pro		
1170	1175	1180
Glu Pro Pro Pro Ser Val Val Arg Phe Ala Val Pro Pro Gly His Thr		
1185	1190	1195 1200
Asn Asn Gly Ser Asp Ser Ser Asp Ser Glu Tyr Ser Ser Gln Thr Thr		
1205	1210	1215
Val Ser Gly Ile Ser Glu Glu Leu Arg Gln Tyr Glu Ala Gln Gln Gly		
1220	1225	1230
Ala Gly Gly Pro Ala His Gln Val Ile Val Glu Ala Thr Glu Asn Pro		
1235	1240	1245
Val Phe Ala Arg Ser Thr Val Val His Pro Asp Ser Arg His Gln Pro		
1250	1255	1260
Pro Leu Thr Pro Arg Gln Gln Pro His Leu Asp Ser Gly Ser Leu Ser		
1265	1270	1275 1280
Pro Gly Arg Gln Gly Gln Gln Pro Arg Arg Asp Pro Pro Arg Glu Gly		
1285	1290	1295
Leu Arg Pro Pro Pro Tyr Arg Pro Arg Arg Asp Ala Phe Glu Ile Ser		
1300	1305	1310
Thr Glu Gly His Ser Gly Pro Ser Asn Arg Asp Arg Ser Gly Pro Arg		
1315	1320	1325
Gly Ala Arg Ser His Asn Pro Arg Asn Pro Thr Ser Thr Ala Met Gly		
1330	1335	1340
Ser Ser Val Pro Ser Tyr Cys Gln Pro Ile Thr Thr Val Thr Ala Ser		
1345	1350	1355 1360
Ala Ser Val Thr Val Ala Val His Pro Pro Pro Gly Pro Gly Arg Asn		
1365	1370	1375

Pro Arg Gly Gly Pro Cys Pro Gly Tyr Glu Ser Tyr Pro Glu Thr Asp  
 1380 1385 1390

His Gly Val Phe Glu Asp Pro His Val Pro Phe His Val Arg Cys Glu  
 1395 1400 1405

Arg Arg Asp Ser Lys Val Glu Val Ile Glu Leu Gln Asp Val Glu Cys  
 1410 1415 1420

Glu Glu Arg Pro Trp Gly Ser Ser Ser Asn  
 1425 1430

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 11 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ile Ile Thr Pro Leu Asp Cys Phe Trp Glu Gly  
 1 5 10

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 5 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Leu Ile Val Gly Gly  
 1 5

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 7 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Pro Phe Phe Trp Glu Gln Tyr  
1 5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GGACGAATTC AARGTNCAYC ARYTNTGG

28

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGACGAATTC CYTCCCARAA RCANTC

26

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGACGAATTC YTNAGANTGYT TYTGCGGA

27

## (2) INFORMATION FOR SEQ ID NO:17:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "primer"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CATACCAGCC AAGCTTGTCN GGCCARTGCA T

31

## (2) INFORMATION FOR SEQ ID NO:18:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5288 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GAATTCGGG GACCGCAAGG AGTGCCGCGG AAGCGCCCGA AGGACAGGCT CGCTCGGCGC	60
GCCGGCTCTC GCTCTTCCGC GAACTGGATG TGGGCAGCGG CGGCCGCAGA GACCTCGGGA	120
CCCCCGCGCA ATGTGGCAAT GGAAGGCGCA GGGTCTGACT CCCCAGCAGC GGCCGCGGCC	180
GCAGCGGCAG CAGCGCCCGC CGTGTGACCA GCAGCAGCGG CTGGTCTGTC AACCAGGAGCC	240
CGAGCCCGAG CAGCCTGCGG CCAGCAGCGT CCTCGCAAGC CGAGCGCCCA GGCGCGCCAG	300
GAGCCCGCAG CAGCGGCAGC AGCGCGCCGG GCCGCCCGGG AAGCCTCCGT CCCCGCGGCG	360
GCGGCGGCGG CGGCGGCGGC AACATGGCCT CGGCTGGTAA CGCCGCCGAG CCCAGGACC	420
GCGGCGGCGG CGGCAGCGGC TGTATCGGTG CCCCAGGACG GCCGGCTGGA GGCGGGAGGC	480
GCAGACGGAC GGGGGGGCTG CGCCGTGCTG CCGCGCCGGA CCGGGACTAT CTGCACCGGC	540
CCAGCTACTG CGACGCGGCC TTCGCTCTGG AGCAGATTTC CAAGGGGAAG GCTACTGGCC	600
GGAAAGCGCC ACTGTGGCTG AGAGCGAAGT TTCAGAGACT CTTATTATAA CTGGGTTGTT	660
ACATTCAAAA AACTGCGGC AAGTTCTTGG TTGTGGGCCT CCTCATATTT GGGGCCTTCG	720

CGGTGGGATT AAAAGCAGCG AACCTCGAGA CCAACGTGGA GGAGCTGTGG GTGGAAGTTG	780
GAGGACGAGT AAGTCGTGAA TTAAATTATA CTCGCCAGAA GATTGGAGAA GAGGCTATGT	840
TTAATCCTCA ACTCATGATA CAGACCCCTA AAGAAGAAGG TGCTAATGTC CTGACCACAG	900
AAGCGCTCCT ACAACACCTG GACTCGGCAC TCCAGGCCAG CCGTGTCCAT GTATACATGT	960
ACAACAGGCA GTGGAAATTG GAACATTTGT GTTACAAATC AGGAGAGCTT ATCACAGAAA	1020
CAGGTTACAT GGATCAGATA ATAGAATATC TTTACCCTTG TTTGATTATT ACACCTTTGG	1080
ACTGCTTCTG GGAAGGGGCG AAATTACAGT CTGGGACAGC ATACCTCCTA GGTAACCTC	1140
CTTTGCGGTG GACAACTTC GACCCTTTGG AATTCCTGGA AGAGTTAAAG AAAATAAACT	1200
ATCAACTGGA CAGCTGGGAG GAAATGCTGA ATAAGGCTGA GGTGGTTCAT GGTACATGG	1260
ACCGCCCCCTG CCTCAATCCG GCCGATCCAG ACTGCCCCGC CACAGCCCCC AACAAAAATT	1320
CAACCAAACC TCTTGATATG GCCCTTGTTT TGAATGGTGG ATGTCATGGC TTATCCAGAA	1380
AGTATATGCA CTGGCAGGAG GAGTTGATTG TGGGTGGCAC AGTCAAGAAC AGCACTGGAA	1440
AACTCGTCAG CGCCCATGCC CTGCAGACCA TGTTCCAGTT AATGACTCCC AAGCAAATGT	1500
ACGAGCACTT CAAGGGGTAC GAGTATGTCT CACACATCAA CTGGAACGAG GACAAAGCGG	1560
CAGCCATCCT GGAGGCCTGG CAGAGGACAT ATGTGGAGGT GGTTCATCAG AGTGTCGCAC	1620
AGAACTCCAC TCAAAGGTG CTTTCCTTCA CCACCACGAC CCTGGACGAC ATCCTGAAAT	1680
CCTTCTCTGA CGTCAGTGTC ATCCGCGTGG CCAGCGGCTA CTTACTCATG CTCGCCTATG	1740
CCTGTCTAAC CATGCTGCGC TGGGACTGCT CCAAGTCCCA GGGTGCCGTG GGGCTGGCTG	1800
GCGTCCTGCT GGTGCACTG TCAGTGGCTG CAGGACTGGG CCTGTGCTCA TTGATCGGAA	1860
TTTCCTTTAA CGTGCAACA ACTCAGGTTT TGCCATTTCT CGCTCTTGGT GTTGGTGTGG	1920
ATGATGTTTT TCTTCTGGCC CACGCCTTCA GTGAAACAGG ACAGAATAAA AGAATCCCTT	1980
TTGAGGACAG GACCGGGGAG TGCCTGAAGC GCACAGGAGC CAGCGTGGCC CTCACGTCCA	2040
TCAGCAATGT CACAGCCTTC TTCATGGCCG CGTTAATCCC AATTCCCGCT CTGCGGGCGT	2100
TCTCCCTCCA GGCAGCGGTA GTAGTGGTGT TCAATTTTGC CATGGTTCTG CTCATTTTTC	2160
CTGCAATTCT CAGCATGGAT TTATATCGAC GCGAGGACAG GAGACTGGAT ATTTTCTGCT	2220
GTTTTACAAG CCCCTGCGTC AGCAGAGTGA TTCAGGTTGA ACCTCAGGCC TACACCGACA	2280
CACACGACAA TACCCGCTAC AGCCCCCAC CTCCCTACAG CAGCCACAGC TTTGCCCATG	2340
AAACGCAGAT TACCATGCAG TCCACTGTCC AGCTCCGCAC GGAGTACGAC CCCCACACGC	2400
ACGTGTACTA CACCACCGCT GAGCCGCGCT CCGAGATCTC TGTGCAGCCC GTCACCGTGA	2460



CACAGGACAC CCTCAGCTGC CAGAGCCCAG AGAGCACCAG CTCCACAAGG GACCTGCTCT	2520
CCCAGTTCTC CGACTCCAGC CTCCACTGCC TCGAGCCCCC CTGTACGAAG TGGACACTCT	2580
CATCTTTTGC TGAGAAGCAC TATGCTCCTT TCCTCTTGAA ACCAAAAGCC AAGGTAGTGG	2640
TGATCTTCCT TTTTCTGGGC TTGCTGGGGG TCAGCCTTTA TGGCACCACC CGAGTGAGAG	2700
ACGGGCTGGA CCTTACGGAC ATTGTACCTC GGGAAACCAG AGAATATGAC TTTATTGCTG	2760
CACAATTCAA ATACTTTTCT TTCTACAACA TGTATATAGT CACCCAGAAA GCAGACTACC	2820
CGAATATCCA GCACTTACTT TACGACCTAC ACAGGAGTTT CAGTAACGTG AAGTATGTCA	2880
TGTTGGAAGA AAACAAACAG CTTCCCAAAA TGTGGCTGCA CTACTTCAGA GACTGGCTTC	2940
AGGGACTTCA GGATGCATTT GACAGTGA CTGAGTGGG GAAATCATG CCAAACAATT	3000
ACAAGAATGG ATCAGACGAT GGAGTCCTTG CCTACAACT CCTGGTGCAA ACCGGCAGCC	3060
GCGATAAGCC CATCGACATC AGCCAGTTGA CTAACACAGC TCTGGTGGAT GCAGATGGCA	3120
TCATTAATCC CAGCGCTTTC TACATCTACC TGACGGCTTG GGTGAGCAAC GACCCCGTCG	3180
CGTATGCTGC CTCCCAGGCC AACATCCGGC CACACCGACC AGAATGGGTC CACGACAAAG	3240
CCGACTACAT GCCTGAAACA AGGCTGAGAA TCCCGGCAGC AGAGCCCATC GAGTATGCCC	3300
AGTTCCCTTT CTACCTCAAC GGGTTGCGGG ACACCTCAGA CTTTGTGGAG GCAATTGAAA	3360
AAGTAAGGAC CATCTGCAGC AACTATACGA GCCTGGGGCT GTCCAGTTAC CCCAACGGCT	3420
ACCCCTTCCT CTTCTGGGAG CAGTACATCG GCCTCCGCCA CTGGCTGCTG CTGTTTCATCA	3480
GCGTGGTGTT GGCCTGCACA TTCCTCGTGT GCGCTGTCTT CTTCTGAAC CCCTGGACGG	3540
CCGGGATCAT TGTGATGGTC CTGGCGCTGA TGACGGTCGA GCTGTTCCGGC ATGATGGGCC	3600
TCATCGGAAT CAAGCTCAGT GCCGTGCCCG TGGTCATCCT GATCGCTTCT GTTGGCATAG	3660
GAGTGGAGTT CACCGTTCAC GTTGCTTTGG CCTTTCTGAC GGCCATCGGC GACAAGAACC	3720
GCAGGGCTGT GCTTGCCCTG GAGCACATGT TTGCACCCGT CCTGGATGGC GCCGTGTCCA	3780
CTCTGCTGGG AGTGCTGATG CTGGCGGGAT CTGAGTTCGA CTTTATTGTC AGGTATTCT	3840
TTGCTGTGCT GGCATCCTC ACCATCCTCG GCGTCTCAA TGGGCTGGTT TTGCTTCCCG	3900
TGCTTTTGTC TTTCTTTGGA CCATATCCTG AGGTGTCTCC AGCCAACGGC TTGAACCGCC	3960
TGCCCCACACC CTCCCCTGAG CCACCCCCCA GCGTGGTCCG CTTGCGCATG CCGCCCGGCC	4020
ACACGCACAG CGGGTCTGAT TCCTCCGACT CGGAGTATAG TTCCAGACG ACAGTGTGAG	4080
GCCTCAGCGA GGAGCTTCGG CACTACGAGG CCCAGCAGGG CGCGGGAGGC CCTGCCACCC	4140
AAGTGATCGT GGAAGCCACA GAAAACCCCG TCTTCGCCCA CTCCACTGTG GTCCATCCCG	4200

AATCCAGGCA TCACCCACCC TCGAACCCGA GACAGCAGCC CCACCTGGAC TCAGGGTCCC 4260  
 TGCCTCCCGG ACGGCAAGGC CAGCAGCCCC GCAGGGACCC CCCCAGAGAA GGCTTGTGGC 4320  
 CACCCCTCTA CAGACCGCGC AGAGACGCTT TTGAAATTTC TACTGAAGGG CATTCTGGCC 4380  
 CTAGCAATAG GGCCCGCTGG GGCCCTCGCG GGGCCCGTTC TCACAACCCCT CGGAACCCAG 4440  
 CGTCCACTGC CATGGGCAGC TCOGTGCCCC GCTACTGCCA GCCCATCACC ACTGTGACGG 4500  
 CTTCTGCCTC CGTGACTGTC GCCGTGCACC CGCCGCCTGT CCCTGGGCCT GGGCGGAACC 4560  
 CCCGAGGGGG ACTCTGCCCA GGCTACCCTG AGACTGACCA CGGCCTGTTT GAGGACCCCC 4620  
 ACGTGCCTTT CCACGTCCGG TGTGAGAGGA GGGATTGCAA GGTGGAAGTC ATTGAGCTGC 4680  
 AGGACCTGGA ATGCGAGGAG AGGCCCCGGG GAAGCAGCTC CAACTGAGGG TGATTAAAAT 4740  
 CTGAAGCAAA GAGGCCAAAG ATTGGAACC CCCCACCCCC ACCTCTTTCC AGAACTGCTT 4800  
 GAAGAGAACT GGTGGAGTT ATGGAAAAGA TGCCCTGTGC CAGGACAGCA GTTCATTGTT 4860  
 ACTGTAACCG ATTGTATTAT TTGTAAAT ATTTCTATAA ATATTTAAGA GATGTACACA 4920  
 TGTGTAATAT AGGAAGGAAG GATGTAAAGT GGTATGATCT GGGGCTTCTC CACTCCTGCC 4980  
 CCAGAGTGTG GAGGCCACAG TGGGGCCTCT CCGTATTTGT GCATTGGGCT CCGTGCCACA 5040  
 ACCAAGCTTC ATTAGTCTTA AATTCAGCA TATGTTGCTG CTGCTTAAAT ATTGTATAAT 5100  
 TTACTTGTAT AATTCTATGC AAATATTGCT TATGTAATAG GATTATTTG TAAAGGTTTC 5160  
 TGTTTAAAT ATTTTAAATT TGCATATCAC AACCTGTGG TAGTATGAAA TGTTACTGTT 5220  
 AACTTTCAA CACGCTATGC GTGATAATTT TTTGTTTAA TGAGCAGATA TGAAGAAAGC 5280  
 CCGGAATT 5288

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1447 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Ala Ser Ala Gly Asn Ala Ala Glu Pro Gln Asp Arg Gly Gly Gly  
 1                      5                      10                      15  
 Gly Ser Gly Cys Ile Gly Ala Pro Gly Arg Pro Ala Gly Gly Gly Arg  
 20                      25                      30

Arg Arg Arg Thr Gly Gly Leu Arg Arg Ala Ala Ala Pro Asp Arg Asp  
 35 40 45  
 Tyr Leu His Arg Pro Ser Tyr Cys Asp Ala Ala Phe Ala Leu Glu Gln  
 50 55 60  
 Ile Ser Lys Gly Lys Ala Thr Gly Arg Lys Ala Pro Leu Trp Leu Arg  
 65 70 75 80  
 Ala Lys Phe Gln Arg Leu Leu Phe Lys Leu Gly Cys Tyr Ile Gln Lys  
 85 90 95  
 Asn Cys Gly Lys Phe Leu Val Val Gly Leu Leu Ile Phe Gly Ala Phe  
 100 105 110  
 Ala Val Gly Leu Lys Ala Ala Asn Leu Glu Thr Asn Val Glu Glu Leu  
 115 120 125  
 Trp Val Glu Val Gly Gly Arg Val Ser Arg Glu Leu Asn Tyr Thr Arg  
 130 135 140  
 Gln Lys Ile Gly Glu Glu Ala Met Phe Asn Pro Gln Leu Met Ile Gln  
 145 150 155 160  
 Thr Pro Lys Glu Glu Gly Ala Asn Val Leu Thr Thr Glu Ala Leu Leu  
 165 170 175  
 Gln His Leu Asp Ser Ala Leu Gln Ala Ser Arg Val His Val Tyr Met  
 180 185 190  
 Tyr Asn Arg Gln Trp Lys Leu Glu His Leu Cys Tyr Lys Ser Gly Glu  
 195 200 205  
 Leu Ile Thr Glu Thr Gly Tyr Met Asp Gln Ile Ile Glu Tyr Leu Tyr  
 210 215 220  
 Pro Cys Leu Ile Ile Thr Pro Leu Asp Cys Phe Trp Glu Gly Ala Lys  
 225 230 235 240  
 Leu Gln Ser Gly Thr Ala Tyr Leu Leu Gly Lys Pro Pro Leu Arg Trp  
 245 250 255  
 Thr Asn Phe Asp Pro Leu Glu Phe Leu Glu Glu Leu Lys Lys Ile Asn  
 260 265 270  
 Tyr Gln Val Asp Ser Trp Glu Glu Met Leu Asn Lys Ala Glu Val Gly  
 275 280 285  
 His Gly Tyr Met Asp Arg Pro Cys Leu Asn Pro Ala Asp Pro Asp Cys  
 290 295 300  
 Pro Ala Thr Ala Pro Asn Lys Asn Ser Thr Lys Pro Leu Asp Met Ala  
 305 310 315 320  
 Leu Val Leu Asn Gly Gly Cys His Gly Leu Ser Arg Lys Tyr Met His  
 325 330 335  
 Trp Gln Glu Glu Leu Ile Val Gly Gly Thr Val Lys Asn Ser Thr Gly

340 345 350  
 Lys Leu Val Ser Ala His Ala Leu Gln Thr Met Phe Gln Leu Met Thr  
 355 360 365  
 Pro Lys Gln Met Tyr Glu His Phe Lys Gly Tyr Glu Tyr Val Ser His  
 370 375 380  
 Ile Asn Trp Asn Glu Asp Lys Ala Ala Ala Ile Leu Glu Ala Trp Gln  
 385 390 395 400  
 Arg Thr Tyr Val Glu Val Val His Gln Ser Val Ala Gln Asn Ser Thr  
 405 410 415  
 Gln Lys Val Leu Ser Phe Thr Thr Thr Thr Leu Asp Asp Ile Leu Lys  
 420 425 430  
 Ser Phe Ser Asp Val Ser Val Ile Arg Val Ala Ser Gly Tyr Leu Leu  
 435 440 445  
 Met Leu Ala Tyr Ala Cys Leu Thr Met Leu Arg Trp Asp Cys Ser Lys  
 450 455 460  
 Ser Gln Gly Ala Val Gly Leu Ala Gly Val Leu Leu Val Ala Leu Ser  
 465 470 475 480  
 Val Ala Ala Gly Leu Gly Leu Cys Ser Leu Ile Gly Ile Ser Phe Asn  
 485 490 495  
 Ala Ala Thr Thr Gln Val Leu Pro Phe Leu Ala Leu Gly Val Gly Val  
 500 505 510  
 Asp Asp Val Phe Leu Leu Ala His Ala Phe Ser Glu Thr Gly Gln Asn  
 515 520 525  
 Lys Arg Ile Pro Phe Glu Asp Arg Thr Gly Glu Cys Leu Lys Arg Thr  
 530 535 540  
 Gly Ala Ser Val Ala Leu Thr Ser Ile Ser Asn Val Thr Ala Phe Phe  
 545 550 555 560  
 Met Ala Ala Leu Ile Pro Ile Pro Ala Leu Arg Ala Phe Ser Leu Gln  
 565 570 575  
 Ala Ala Val Val Val Val Phe Asn Phe Ala Met Val Leu Leu Ile Phe  
 580 585 590  
 Pro Ala Ile Leu Ser Met Asp Leu Tyr Arg Arg Glu Asp Arg Arg Leu  
 595 600 605  
 Asp Ile Phe Cys Cys Phe Thr Ser Pro Cys Val Ser Arg Val Ile Gln  
 610 615 620  
 Val Glu Pro Gln Ala Tyr Thr Asp Thr His Asp Asn Thr Arg Tyr Ser  
 625 630 635 640  
 Pro Pro Pro Pro Tyr Ser Ser His Ser Phe Ala His Glu Thr Gln Ile  
 645 650 655

Thr Met Gln Ser Thr Val Gln Leu Arg Thr Glu Tyr Asp Pro His Thr  
 660 665 670  
 His Val Tyr Tyr Thr Thr Ala Glu Pro Arg Ser Glu Ile Ser Val Gln  
 675 680 685  
 Pro Val Thr Val Thr Gln Asp Thr Leu Ser Cys Gln Ser Pro Glu Ser  
 690 695 700  
 Thr Ser Ser Thr Arg Asp Leu Leu Ser Gln Phe Ser Asp Ser Ser Leu  
 705 710 715 720  
 His Cys Leu Glu Pro Pro Cys Thr Lys Trp Thr Leu Ser Ser Phe Ala  
 725 730 735  
 Glu Lys His Tyr Ala Pro Phe Leu Leu Lys Pro Lys Ala Lys Val Val  
 740 745 750  
 Val Ile Phe Leu Phe Leu Gly Leu Leu Gly Val Ser Leu Tyr Gly Thr  
 755 760 765  
 Thr Arg Val Arg Asp Gly Leu Asp Leu Thr Asp Ile Val Pro Arg Glu  
 770 775 780  
 Thr Arg Glu Tyr Asp Phe Ile Ala Ala Gln Phe Lys Tyr Phe Ser Phe  
 785 790 795 800  
 Tyr Asn Met Tyr Ile Val Thr Gln Lys Ala Asp Tyr Pro Asn Ile Gln  
 805 810 815  
 His Leu Leu Tyr Asp Leu His Arg Ser Phe Ser Asn Val Lys Tyr Val  
 820 825 830  
 Met Leu Glu Glu Asn Lys Gln Leu Pro Lys Met Trp Leu His Tyr Phe  
 835 840 845  
 Arg Asp Trp Leu Gln Gly Leu Gln Asp Ala Phe Asp Ser Asp Trp Glu  
 850 855 860  
 Thr Gly Lys Ile Met Pro Asn Asn Tyr Lys Asn Gly Ser Asp Asp Gly  
 865 870 875 880  
 Val Leu Ala Tyr Lys Leu Leu Val Gln Thr Gly Ser Arg Asp Lys Pro  
 885 890 895  
 Ile Asp Ile Ser Gln Leu Thr Lys Gln Arg Leu Val Asp Ala Asp Gly  
 900 905 910  
 Ile Ile Asn Pro Ser Ala Phe Tyr Ile Tyr Leu Thr Ala Trp Val Ser  
 915 920 925  
 Asn Asp Pro Val Ala Tyr Ala Ala Ser Gln Ala Asn Ile Arg Pro His  
 930 935 940  
 Arg Pro Glu Trp Val His Asp Lys Ala Asp Tyr Met Pro Glu Thr Arg  
 945 950 955 960  
 Leu Arg Ile Pro Ala Ala Glu Pro Ile Glu Tyr Ala Gln Phe Pro Phe

965	970	975
Tyr Leu Asn Gly Leu Arg Asp Thr Ser Asp Phe Val Glu Ala Ile Glu 980 985 990		
Lys Val Arg Thr Ile Cys Ser Asn Tyr Thr Ser Leu Gly Leu Ser Ser 995 1000 1005		
Tyr Pro Asn Gly Tyr Pro Phe Leu Phe Trp Glu Gln Tyr Ile Gly Leu 1010 1015 1020		
Arg His Trp Leu Leu Leu Phe Ile Ser Val Val Leu Ala Cys Thr Phe 1025 1030 1035 1040		
Leu Val Cys Ala Val Phe Leu Leu Asn Pro Trp Thr Ala Gly Ile Ile 1045 1050 1055		
Val Met Val Leu Ala Leu Met Thr Val Glu Leu Phe Gly Met Met Gly 1060 1065 1070		
Leu Ile Gly Ile Lys Leu Ser Ala Val Pro Val Val Ile Leu Ile Ala 1075 1080 1085		
Ser Val Gly Ile Gly Val Glu Phe Thr Val His Val Ala Leu Ala Phe 1090 1095 1100		
Leu Thr Ala Ile Gly Asp Lys Asn Arg Arg Ala Val Leu Ala Leu Glu 1105 1110 1115 1120		
His Met Phe Ala Pro Val Leu Asp Gly Ala Val Ser Thr Leu Leu Gly 1125 1130 1135		
Val Leu Met Leu Ala Gly Ser Glu Phe Asp Phe Ile Val Arg Tyr Phe 1140 1145 1150		
Phe Ala Val Leu Ala Ile Leu Thr Ile Leu Gly Val Leu Asn Gly Leu 1155 1160 1165		
Val Leu Leu Pro Val Leu Leu Ser Phe Phe Gly Pro Tyr Pro Glu Val 1170 1175 1180		
Ser Pro Ala Asn Gly Leu Asn Arg Leu Pro Thr Pro Ser Pro Glu Pro 1185 1190 1195 1200		
Pro Pro Ser Val Val Arg Phe Ala Met Pro Pro Gly His Thr His Ser 1205 1210 1215		
Gly Ser Asp Ser Ser Asp Ser Glu Tyr Ser Ser Gln Thr Thr Val Ser 1220 1225 1230		
Gly Leu Ser Glu Glu Leu Arg His Tyr Glu Ala Gln Gln Gly Ala Gly 1235 1240 1245		
Gly Pro Ala His Gln Val Ile Val Glu Ala Thr Glu Asn Pro Val Phe 1250 1255 1260		
Ala His Ser Thr Val Val His Pro Glu Ser Arg His His Pro Pro Ser 1265 1270 1275 1280		

Asn Pro Arg Gln Gln Pro His Leu Asp Ser Gly Ser Leu Pro Pro Gly  
 1285 1290 1295  
 Arg Gln Gly Gln Gln Pro Arg Arg Asp Pro Pro Arg Glu Gly Leu Trp  
 1300 1305 1310  
 Pro Pro Leu Tyr Arg Pro Arg Arg Asp Ala Phe Glu Ile Ser Thr Glu  
 1315 1320 1325  
 Gly His Ser Gly Pro Ser Asn Arg Ala Arg Trp Gly Pro Arg Gly Ala  
 1330 1335 1340  
 Arg Ser His Asn Pro Arg Asn Pro Ala Ser Thr Ala Met Gly Ser Ser  
 1345 1350 1355 1360  
 Val Pro Gly Tyr Cys Gln Pro Ile Thr Thr Val Thr Ala Ser Ala Ser  
 1365 1370 1375  
 Val Thr Val Ala Val His Pro Pro Pro Val Pro Gly Pro Gly Arg Asn  
 1380 1385 1390  
 Pro Arg Gly Gly Leu Cys Pro Gly Tyr Pro Glu Thr Asp His Gly Leu  
 1395 1400 1405  
 Phe Glu Asp Pro His Val Pro Phe His Val Arg Cys Glu Arg Arg Asp  
 1410 1415 1420  
 Ser Lys Val Glu Val Ile Glu Leu Gln Asp Val Glu Cys Glu Glu Arg  
 1425 1430 1435 1440  
 Pro Arg Gly Ser Ser Ser Asn  
 1445

WHAT IS CLAIMED IS:

1. A DNA sequence other than present in a chromosome encoding a *patched* gene other than the *Drosophila patched* gene or fragment thereof of at least about 12bp  
5 different from the sequence of the *Drosophila patched* gene.
2. A DNA sequence according to Claim 1, wherein said *patched* gene is a mammalian gene.
- 10 3. A DNA sequence according to Claim 1 for human, mouse, mosquito, butterfly or beetle *patched* gene.
4. A DNA sequence according to Claim 3, wherein said DNA sequence is a human sequence.
- 15 5. A DNA sequence according to Claim 4, wherein said DNA sequence is a mouse sequence.
6. A DNA sequence according to Claim 1, wherein said DNA sequence is a  
20 fragment of at least about 18bp.
7. A DNA sequence according to Claim 1 joined to a DNA sequence comprising a restriction enzyme recognition sequence.
- 25 8. An expression cassette comprising a transcriptional initiation region functional in an expression host, a DNA sequence according to Claim 1 under the transcriptional regulation of said transcriptional initiation region, and a transcriptional termination region functional in said expression host.
- 30 9. An expression cassette according to Claim 8, wherein said transcriptional initiation region is heterologous to said DNA sequence according to Claim 1.



10. An expression cassette according to Claim 8, wherein said transcriptional initiation region is homologous to said DNA sequence according to Claim 1 and includes the enhancer region.
- 5 11. A cell comprising an expression cassette according to Claim 8 as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of said expression cassette into said host cell and the cellular progeny of said host cell.
- 10 12. A cell according to Claim 11, further comprising the *patched* protein in the cellular membrane of said cell.
13. A cell according to Claim 11, wherein said *patched* protein is a mouse *patched* protein.
- 15 14. A cell according to Claim 11, wherein said *patched* gene is a human *patched* protein.
15. A cell according to Claim 11, wherein said transcriptional initiation region is a
- 20 *Drosophila patched* gene transcriptional initiation region comprising the promoter and enhancer joined to a heterologous gene.
16. A cell comprising an expression cassette comprising a transcriptional initiation region functional in an expression host, said transcriptional initiation region
- 25 consisting of a 5' non-coding region regulating the transcription of *patched* protein comprising the promoter and enhancer, a marker gene, and a transcriptional termination region, as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of said expression cassette into said host, and the cellular progeny thereof.
- 30

17. A cell according to Claim 16, wherein said transcriptional initiation region is the *Drosophila* region.
18. A method for following embryonic development employing the *patched* protein in an embryo, said method comprising:
- 5 integrating an expression cassette comprising a transcriptional initiation region functional in embryonic host cells, said transcriptional initiation region consisting of a 5' non-coding region regulating the transcription of *patched* protein, a marker gene, and a transcriptional termination region, wherein said embryonic host cells are
- 10 capable of developing into a fetus;
- growing said embryonic host cells, whereby proliferation and differentiation occur; and
- locating cells comprising expression of the *patched* protein by means of expression of said marker gene.
- 15
19. A method for producing *patched* protein, said method comprising:
- growing a cell according to Claim 11, whereby said *patched* protein is expressed; and
- isolating said *patched* protein free of other proteins.
- 20
20. A method for screening candidate compounds for binding affinity to the *patched* protein, said method comprising:
- combining said candidate protein with a vertebrate or invertebrate cell comprising said *patched* protein in the membrane of said cell and an expression
- 25 cassette comprising a transcriptional initiation region functional in said cell, a DNA sequence according to Claim 1 comprising the entire coding sequence under the transcriptional regulation of said transcriptional initiation region, and a transcriptional termination region functional in said cell, expressing said *patched* protein in said cell; and
- 30 assaying for the binding of said candidate compound to said *patched* protein.

21. A method for screening candidate compounds for agonist activity with the *patched* protein, said method comprising:
- combining said candidate protein with a vertebrate or invertebrate cell comprising said *patched* protein in the membrane of said cell and an expression
- 5 cassette comprising a transcriptional initiation region functional in an expression host, said transcriptional initiation region consisting of a 5' non-coding region regulating the transcription of *patched* protein, a marker gene, and a transcriptional termination region, as part of an extrachromosomal element or integrated into the genome of a host cell; and
- 10 assaying for the expression of said marker gene.
22. A monoclonal antibody binding specifically to a *patched* protein, other than the *Drosophila patched* protein.

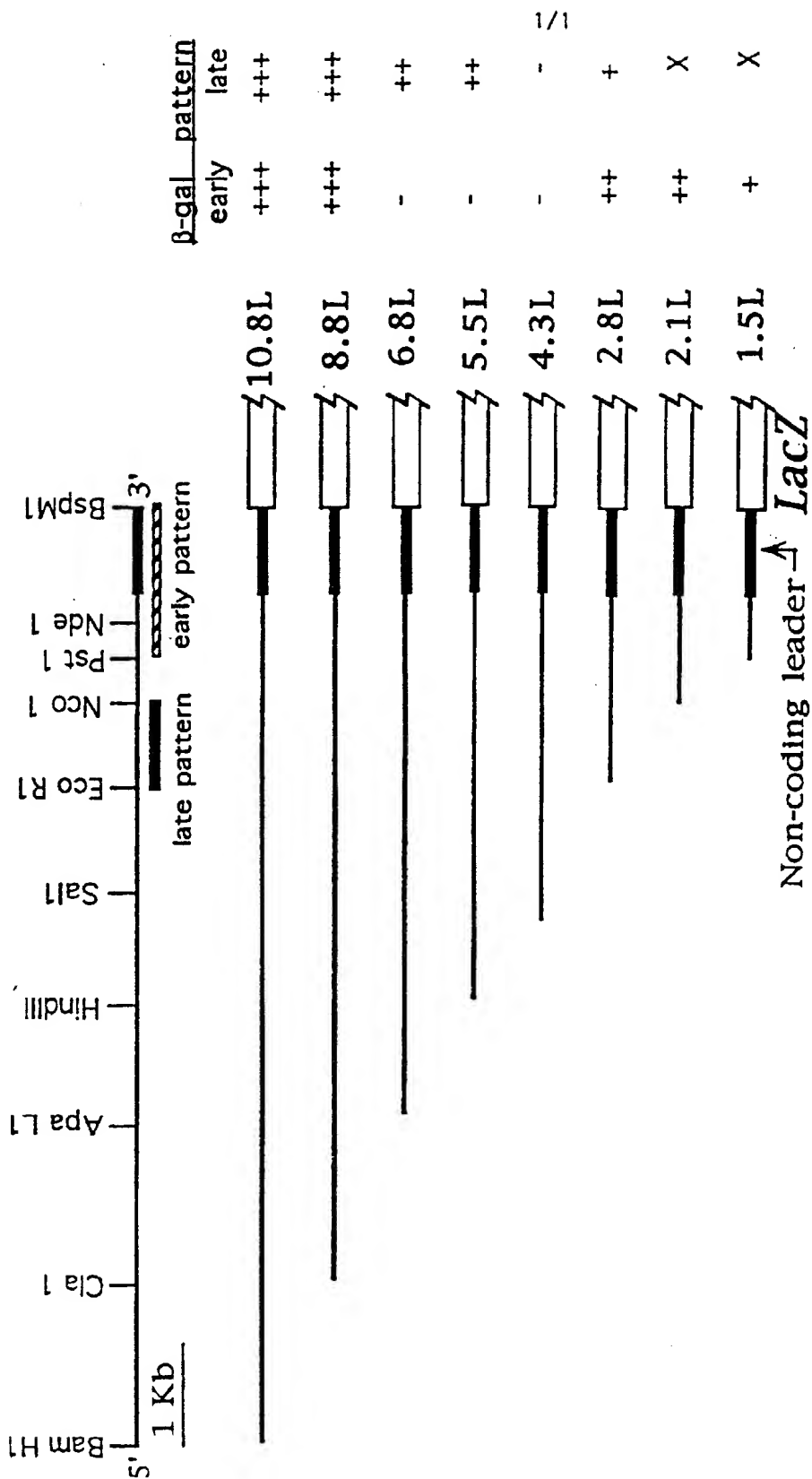


FIGURE 1

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/13233

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : C12N 5/00, 15/00; C07H 21/00

US CL : 536/23.1; 435/69.1, 172.3, 240.2, 320.1

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1; 435/69.1, 172.3, 240.2, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Nature, Volume 341, issued 12 October 1989, Nakano et al., "A protein with several possible membrane-spanning domains encoded by the Drosophila segment polarity gene patched", pages 508-513, see the entire document.	1-10 and 18
Y	Cell, Volume 59, issued 17 November 1989, Hooper et al., "The Drosophila patched gene encodes a putative membrane protein required for segmental patterning", pages 751-765, see the entire document.	1-10 and 18
Y	Gene, Volume 112, issued 1992, Chavrier et al., "The complexity of the Rab and Rho GTP-binding protein subfamilies revealed by a PCR cloning approach", pages 261-264, see the entire document.	1-10

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	* T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
* A		document defining the general state of the art which is not considered to be of particular relevance
* E		earlier document published on or after the international filing date
* L		document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reasons (as specified)
* O		document referring to an oral disclosure, use, exhibition or other means
* P		document published prior to the international filing date but later than the priority date claimed
	* X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
	* Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
	* &	document member of the same patent family

Date of the actual completion of the international search

19 JANUARY 1996

Date of mailing of the international search report

31 JAN 1996

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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US95/13233

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Biochemistry, Volume 31, No. 44, issued 1992, Ma et al., "Molecular cloning and characterization of rKlk10, a cDNA encoding T-kininogenase from rat submandibular gland and kidney", pages 10922-10928, see the experimental procedures on page 10923.	1-10
Y	Gene, Volume 74, issued 1988, Thummel et al., "Vectors for Drosophila P-element-mediated transformation and tissue culture transfection", pages 445-456, see the entire document.	8-10 and 18
Y	Developmental Genetics, Volume 12, issued 1991, Perrimon et al., "Generating lineage-specific markers to study Drosophila development", pages 238-252, see the entire document.	8-10 and 18

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US95/13233

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-10 AND 18

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US95/13233

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, DIALOG

search terms: patched, gene, cloning, PCR, human, mammalian, mouse, mosquito, butterfly, beetle, DNA, drosophila, embryo, gal, galactosidase, develop, review, inventors' names

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-10 and 18, drawn to a DNA sequence encoding a patched gene, an expression cassette, and a method for following embryonic development by integrating the expression cassette.

Group II, claims 11-17 and 19, drawn to a cell and a method for producing patched protein by growing the cell.

Group III, claim 20, drawn to a method for screening candidate compounds for binding affinity to the patched protein.

Group IV, claim 21, drawn to a method for screening candidate compounds for agonist activity with the patched protein.

Group V, claim 22, drawn to a monoclonal antibody specific for a patched protein.

The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1, because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups I, II and V are not so linked as to form a single inventive concept because the DNA sequence of Group I, the cell of Group II and the monoclonal antibody of Group V are drawn to three different products.

Groups II and III are not so linked as to form a single inventive concept because they are drawn to materially different methods. The method of Group II involves growing a cell while the method of Group III involves combining a candidate compound with a cell and then assaying for binding.

Groups II and IV are not so linked as to form a single inventive concept because they are drawn to materially different methods. The method of Group II involves growing a cell while the method of Group IV involves combining a candidate compound with a cell and then assaying for expression of a marker gene.